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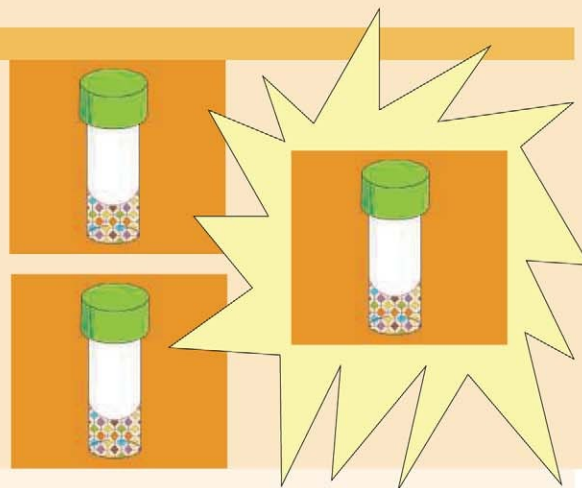
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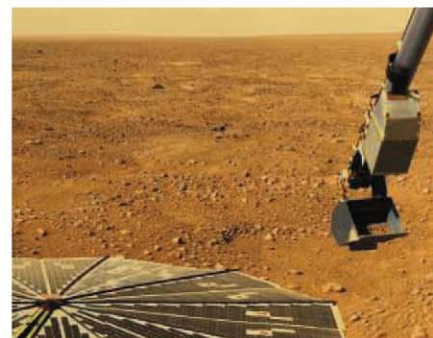
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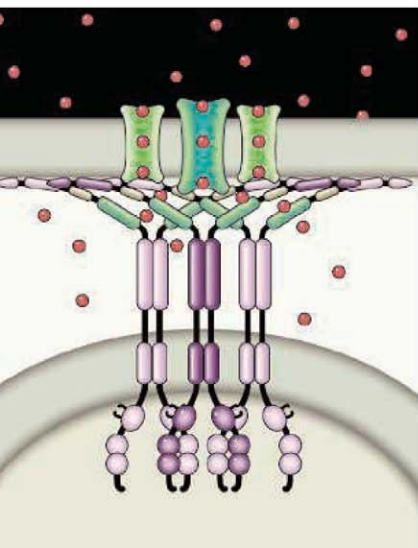
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An artist's rendition of the Kepler-9 system in which two Saturn-size planets transit the same star. The radii of the planets are roughly 8% of the radius of the star, which itself is 10% larger in radius than our Sun. Timing variations in the transits of these planets provide a signature of their gravitational interaction and can be used to establish their masses. See page 51.

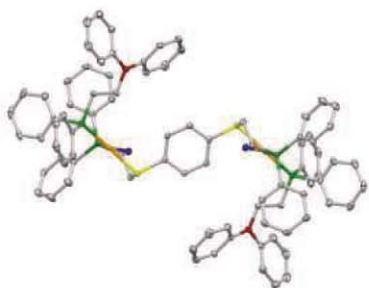
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A fossil penguin shows that the wing and feather form evolved before distinctive microstructural changes in the feathers.

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Evidence for a Collective Intelligence Factor in the Performance of Human Groups

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Structure of a Eukaryotic CLC Transporter Defines an Intermediate State in the Transport Cycle

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Full text at www.sciencemag.org/cgi/content/full/330/6000/35-a

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S. S. Cash et al.

Full text at www.sciencemag.org/cgi/content/full/330/6000/35-b

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RESEARCH ARTICLE: Signaling from the Endoplasmic Reticulum Activates Brassinosteroid Signaling and Promotes Acclimation to Stress in *Arabidopsis*

P. Che et al.

BR signaling may integrate stress responses and growth processes to optimize growth under challenging environmental conditions.

RESEARCH ARTICLE: Coupling Mechanism of a GPCR and a Heterotrimeric G Protein During Chemoattractant Gradient Sensing in *Dictyostelium*

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RESEARCH ARTICLE: Molecular Mechanism of Calcium Channel Regulation in the Fight-or-Flight Response

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PERSPECTIVE: Sphingolipids—The Oil on the TRAFire That Promotes Inflammation

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SCIENCE CAREERS

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B. L. Benderly

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A. M. Boccardo

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PERSPECTIVE: Bacterial Exploitation of Host Cell Signaling

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RESEARCH ARTICLE: Interfering with Resistance to Smoothed Antagonists by Inhibition of the PI3K Pathway in Medulloblastoma

S. Buonamici et al.

Resistance of medulloblastoma to Smo antagonists can be delayed or prevented by specific drug combinations.

RESEARCH ARTICLE: A Central Role for Free Heme in the Pathogenesis of Severe Sepsis

R. Larsen et al.

Heme from red blood cells released in septic shock worsens organ dysfunction and increases the risk of death, but can be overcome by a scavenger of free heme.

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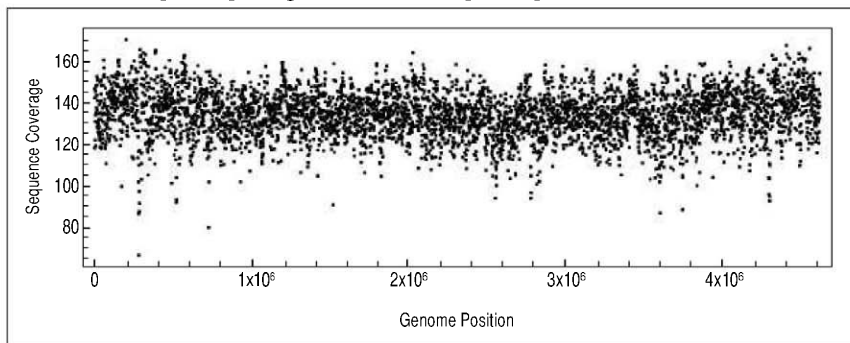
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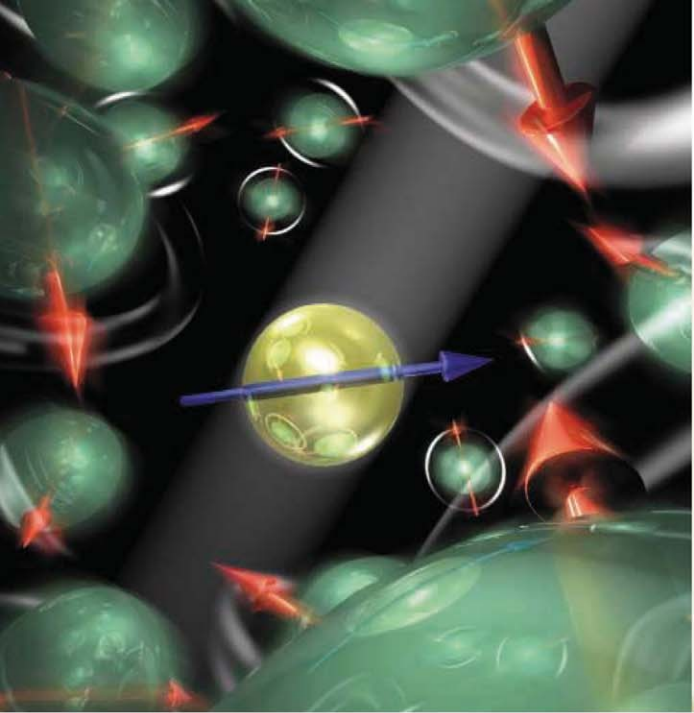
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Single electron spins in solid-state environments have been explored as candidates for quantum information storage and computation; however, they often interact strongly with their surroundings and lose the stored information on the time scale of pico- to milliseconds. Dynamical decoupling schemes have been introduced to “undo” the effects of this interaction by applying a sequence of control pulses that reverse the undesirable evolution of the system. **De Lange *et al.*** (p. 60, published online 9 September) tested several decoupling schemes on a nitrogen vacancy center in diamond and found that a scheme with evenly spaced pulses with double-axis decoupling could prolong the coherence time of an arbitrary spin state up to 25-fold.

Extra Exoplanet?

A planet is said to transit its star if it can be seen to pass in front of the star; 19% of the known extrasolar planets are transiting planets. A lone planet will transit in an exactly periodic manner; if other planets are present, however, variations in transit duration are expected because of gravitational interactions. **Holman *et al.*** (p. 51, published online 26 August; see the cover; see the Perspective by **Laughlin**) report timing variations in the transits of two exoplanets detected by the Kepler space telescope. The planets have masses similar to that of Saturn and transit the same Sun-like star. A third planet several times the mass of Earth may also transit the star in an interior orbit.

Smoke Gets in Your Lungs

Chronic obstructive pulmonary disease (COPD) is a leading cause of death in the United States, primarily caused by cigarette smoking. The chronic inflammation that leads to tissue damage and organ dysfunction in COPD is mediated in large part by neutrophils, a type of granulocytic immune cell. **Snelgrove *et al.*** (p. 90, published online 2 September; see the Perspective by **Barnes**) now provide an explanation for why neutrophils stick around in the lung during COPD. The neutrophil chemoattractant Pro-Gly-Pro (PGP) is a biomarker for COPD and promotes neutrophil accumulation. The enzyme leukotriene A_4 hydrolase degrades PGP in mice, and its activity was reduced by cigarette smoke both in vivo and in vitro. In contrast, during acute influenza infection in mice, leukotriene A_4 hydrolase functioned normally, allowing for PGP degradation and the resolution of inflammation. Thus, in COPD, cigarette smoking may lead to the

accumulation PGP—which, in turn, could keep neutrophils in the lung to drive inflammation and subsequent lung damage and dysfunction.

New Guinea's Ancient Colonies

Isolated by water, Australia and New Guinea were some of the last major parts of the world colonized by modern humans. **Summerhayes *et al.*** (p. 78; see the Perspective by **Gosden**) describe an archaeological site in the highlands of New Guinea that sheds light on this migration. The record extends back to nearly 50,000 years ago and thus represents one of the earliest known records. Nuts and yams were widely consumed, and the variety of stone tools discovered implies that the early humans may have cleared forest patches to promote the growth of useful plants.



Two for One

Solar cells often contain materials that absorb a broad spectrum of light above a certain frequency threshold, or band gap. Unfortunately, much of the energy contained in this light is wasted, because any balance exceeding the band gap tends to be dissipated as heat, rather than harnessed into electric current. Recent spectroscopic studies have shown that incident photons with energy several multiples of the band gap can transiently generate more than one current carrier, but the excess carriers tend to collapse before they can be diverted into the

circuit. **Sambur *et al.*** (p. 63) now show that, when light-absorbing lead sulfide nanoparticles are carefully coupled to smoothly polished titanium dioxide crystalline electrodes, such excess carriers can be transferred into the circuit before collapsing.

Toward Microbial Taxol

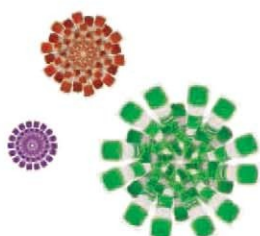
Taxol, a minor chemical constituent of yew tree bark, has provided a potent cancer treatment. Production methods presently rely on plant cell cultures. **Ajikumar *et al.*** (p. 70; see the Perspective by **Liu and Khosla**) engineered *Escherichia coli* cells to produce a key taxol precursor, in which the polycyclic carbon skeleton is intact. The approach relied on optimizing the relative activity of two pathways, the first of which synthesized isoprenoid building blocks that were then stitched together with the second pathway. Accumulation of indole as a by-product inhibited the isoprenoid pathway—an insight that should facilitate more efficient engineered biosynthesis of a wide range of commercially important isoprenoid derivatives.

Indirect Oxidation by Oxygen

Partial oxidation of alcohols to aldehydes and ketones must avoid complete oxidation to carbon dioxide and water. **Zope *et al.*** (p. 74) examined the partial oxidation of ethanol and glycerol to acids in alkaline aqueous solvents over gold and platinum catalysts. Conversions were highest for gold supported on titania, but studies with isotopically labeled molecular oxygen showed that

Continued on page 10

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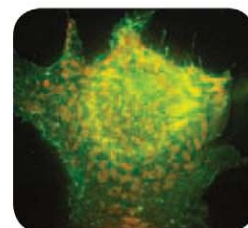
oxygen incorporated into the acid comes from hydroxide ions. Direct incorporation of oxygen did not occur even for the platinum catalysts, despite the fact that oxygen can dissociate on this metal. Instead, molecular oxygen appeared to regenerate hydroxide ions at the metal surface through the formation of peroxide intermediates.

Auroral Chorus

Energetic particles that arrive from near-Earth space produce photon emissions—the aurora—as they bombard the atmosphere in the polar regions. The pulsating aurora, which is characterized by temporal intensity variations, is thought to be caused by modulations in electron precipitation possibly produced by resonance with electromagnetic waves in Earth's magnetosphere. **Nishimura *et al.*** (p. 81) present a detailed study of an event that showed a good correlation between the temporal changes in auroral luminosity and chorus emission—a type of electromagnetic wave occurring in Earth's magnetosphere. The results points to chorus waves as the driver of the pulsating aurora.

Channel STIMulation

The STIM1 protein functions as a calcium sensor and regulates entry of calcium into cells across the plasma membrane. When cell surface receptors are stimulated and cause release of calcium from internal stores in the endoplasmic reticulum (ER), STIM proteins in the ER membrane interact with the Orai channel pore protein in the plasma membrane to allow calcium entry from the outside of the cell (see the Perspective by **Cahalan**). **Park *et al.*** (p. 101) and **Wang *et al.*** (p. 105) now show that STIM also acts to suppress conductance by another calcium channel—the voltage-operated $\text{Ca}_v1.2$ channel. STIM1 appeared to interact directly with $\text{Ca}_v1.2$ channels in multiple cell types, including vascular smooth muscle cells, neurons, and cultured cells derived from T lymphocytes. The interaction inhibited opening of the $\text{Ca}_v1.2$ channels and caused depletion of the channel from the cell surface.



Improving Yeast for Biofuel Production

The biofuels industry uses the yeast *Saccharomyces cerevisiae* to produce ethanol from sugars derived from cornstarch or sugar cane. Plant cell walls are an attractive sugar source; however, yeast does not grow efficiently on cellulose-derived sugars (cellodextrins). **Galazka *et al.*** (p. 84, published online 9 September) now show that a model cellolytic fungus *Neurospora crassa* relies on a cellodextrin transport system to facilitate growth on cellulose. Yeast reconstituted with this transport system grew efficiently on cellodextrins, which could potentially improve the efficiency of cellulosic biofuel production.

Gaining from a Loss

Mitotic recombination can cause a cell carrying heterozygous mutations in a tumor suppressor gene to lose the wild-type copy of the gene, setting the cell on the pathway to uncontrolled growth. But can mitotic recombination have beneficial effects in other settings—that is, lead to phenotypic correction of a diseased cell by facilitating loss of the disease-causing mutation? **Choate *et al.*** (p. 94, published online 26 August; see the Perspective by **Davis and Candotti**) now find evidence for this type of event in a rare skin disease called ichthyosis with confetti (IWC). Patients with IWC display severe scaling of the skin but have widespread patches of normal skin that reflect clonal expansion of revertant cells. The revertant cells showed loss of heterozygosity on chromosome 17q and, as a result of mitotic recombination, these cells selectively lost dominant disease-causing mutations in the keratin 10 gene (*KRT10*), but retained the wild-type copy of the gene.

One, Two, Three, Remember Me

When a stimulus (such as a word or a face) is presented for the second, third, or fourth time, do the neural representations differ? And, if they do, are multiply represented stimuli remembered better? These questions and related ones have fascinated psychologists for decades, but only recently has it become feasible to begin tackling them using neuroimaging. **Xue *et al.*** (p. 97, published online 9 September) provide evidence that the greater the similarity in the patterns of neural activity during encoding of the item, the greater the likelihood that the item will be remembered.

CREDIT: WANG ET AL.



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The Future of Russian Science

IN MAY, RUSSIAN PRIME MINISTER VLADIMIR PUTIN ADDRESSED THE GENERAL MEETING OF THE Russian Academy of Sciences (RAS), describing plans to overhaul the country's science and innovation agenda and structure, with the goal of bolstering Russia's economy and global competitiveness. A major question is what role the RAS—the largest and most prestigious scientific institution in Russia—will have in these projects.

The RAS's prestige is grounded in a rich history of major discoveries by its scientists, in the thousands of Ph.D.'s it has trained, in the Russian Nobel Prize winners who did their seminal work there, and in its many outstanding scientists and scholars who became professors at various universities. Although recently the RAS has been attacked in both the Western and Russian press for low efficiency, it is hard to imagine how other organizations could assume these multifaceted functions in the foreseeable future. Currently, there are plans to expand universities and research centers and to integrate research, education, and production activities through mechanisms that fail to include any increased investment in the RAS, which received less funding in 2010 than in 2009. Yet a major reason for RAS's low efficiency is the despicable level of funding it has received for several decades, and it is difficult to see how its more than 50,000 scientists can help to drive the nation's transformation agenda without serious new support.

Consider the situation with respect to molecular biology. In Soviet times, the RAS maintained a rather strong effort in molecular biology research. But this almost perished after a sharp decrease in financial support in 1990 that triggered a massive emigration of well-educated experienced specialists. However, a number of strong groups managed to survive, largely by acquiring outside grants and establishing collaborations with Western research groups. After the funding of science in Russia began to increase again, the Presidium of the RAS created the program Molecular and Cellular Biology in 2003. This program supported about 100 of the best Russian research groups with awards of up to \$150,000 per year. The competition was open to scientists who had emigrated, and many young researchers preferred to stay in Russia or even return to join this effort. The recipients of these awards were selected mainly on the basis of publications in highly rated international journals; as a result, between 2003 and 2009, the program produced about 2000 papers in peer-reviewed international journals, including the most reputable ones. Such results demonstrate that providing sufficient funding for strong groups can revive effective scientific activity at RAS institutions throughout Russia. It is therefore disappointing that support for the program was reduced because of inflation over the years, and then suddenly cut by about a third in 2010, which could nullify all previous efforts.

Producing outstanding basic experimental science requires three pillars: talented scientists, adequate equipment, and favorable conditions for a researcher's work and life. Many talented scientists still remain in Russia. However, the outmoded equipment in many institutes, low salaries, and housing problems have been forcing many of the most talented to leave the country. Admittedly, the RAS has suffered in the past from many shortcomings, such as a low percentage of competitively distributed funding and the lack of an efficient mechanism for selecting those research groups that are most worthy of support. But these shortcomings can be fixed through rather simple reforms. At the same time, the RAS can serve as an interface between science and industry to further drive the development of Russian society. In his talk, the prime minister emphasized several key points relevant to the role of the RAS in his plans for improving the nation's innovation capacity. Among them: "The Academy has always been and must remain a key institution of national and social development ... the field of science ... is based on a principle of ... competition ... scientists should have the opportunity to work at state-of-the-art research centers." These are the right strategic ideas, and they should be implemented by building on RAS capacities.

— Georgii Georgiev and Eugene Sverdlov



CHEMISTRY

Breaking Methanol

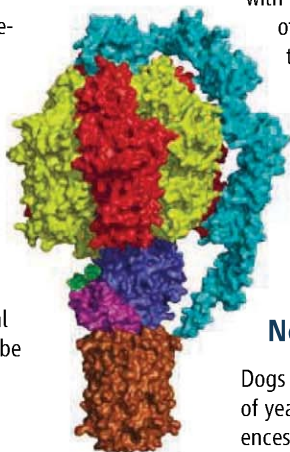
Titanium dioxide (TiO_2) is a highly abundant material, and as such has been heavily investigated for widespread use of its photocatalytic properties. One potential application of increasing importance is the direct use of sunlight to generate hydrogen from water, a reaction that proceeds more readily at TiO_2 surfaces when some methanol is mixed in. In this context, Zhou *et al.* have explored the behavior of methanol monolayers and submonolayers on TiO_2 under ultraviolet irradiation. Using both two-photon photoemission spectroscopy and scanning tunneling microscopy, the authors present evidence for photoinduced dissociation of the adsorbed methanol via H transfer from the $\text{CH}_3\text{O-H}$ bond to a bridge-bonded oxygen on the TiO_2 surface. Accompanying density functional calculations support assignment of the photoemission signal to perturbation of Ti d-orbitals by virtue of the tetravalent metal ion's interaction with the CH_3O radical co-product, which pushes the ion out of plane. The temporal profile of the emission was not mono-exponential, implicating heterogeneous kinetics. — JSY

Chem. Sci. **1**, 10.1039/c0sc00316f (2010).

BIOCHEMISTRY

Flexible Exchange Rates

The membrane-bound enzyme F-ATPase serves an important function in almost all walks of life. It makes ATP (from ADP and inorganic phosphate) by converting the passage of protons down an electrochemical gradient first into mechanical energy and thence into chemical energy. The parts of the enzyme (red and yellow) that bind ADP lie mostly outside the membrane, and three such binding sites are converted sequentially from open to loose to tight conformations. The energy contained in the movement of protons is extracted by forcing them through a membrane-embedded ring (brown) of identical subunits, each of which captures a single proton on a glutamate residue. Turning this ring rotates a central stalk (blue, purple, and green) at 6000 rpm, which drives the cycle of conformational changes. Watt *et al.* describe the crystal structure of the mitochondrial ring and show that it contains eight



Bird in the spotlight.

ECOLOGY

'Round Midnight

Increasingly, artificial light sources are found on the edges of urban areas, and Kempnaers *et al.* have investigated the effect of nighttime illumination on bird behavior and reproduction. From a 7-year study of the reproductive behavior of blue tits, they found that female birds laid eggs on average 1.5 days earlier when living in regions exposed to artificial lighting and that this effect was strongest in the birds nearest to the light sources. In addition, males started singing earlier in the pre-dawn hours, especially if they were younger, and males in lighted territories were more successful at extra-pair matings. The full human impact on the behavioral ecology of these blue tits is not known, but the authors conclude that it may result in differential selection for indicators of good genes among populations whose effects cannot yet be determined. — LMZ

Curr. Biol. **20**, 10.1016/j.cub.2010.08.028 (2010).

subunits, leading to a ratio of eight protons for three ATPs synthesized. What is puzzling is the range of stoichiometries documented thus far, with 10 to 15 subunits found in the F-ATPases of fungi, bacteria, and chloroplasts. All of these enzymes possess the canonical trio of ADP-binding sites, which means that the cost of each ATP varies from as little as 2.7 protons in animals to as many as 5 in microbes. — GJC

Proc. Natl. Acad. Sci. U.S.A. **107**, 16823 (2010).

GENETICS

Not a Dog's Breakfast After All

Dogs have been our companions for thousands of years. This long allegiance and our preferences for dogs with certain traits have led to an

unmatched degree of morphological variation and an opportunity for geneticists to unravel how genes interact with selection to shape phenotypes. Recent work in this area has revealed much about the genes underlying specific traits, such as coat color, foreshortened limbs, and hairlessness. Boyko *et al.* have examined variation at over 60,000 single-nucleotide polymorphisms in 80 dog breeds, African village dogs, and wolves. Interestingly, they found that the majority of variation among breeds was generated by just a few genomic regions of large effect. For example, they identified only six regions associated with body size, and ear floppiness was associated with just a single region. They hypothesize that the small number of regions controlling such a large degree of variation is probably due to the repeated bottlenecks dogs went through in their evolution, first

when they were domesticated and then during breed development. Genetic diversity measures support this hypothesis, as all breeds displayed much lower genetic diversity than either wolves or village dogs. — SNV

PLoS Biol. **8**, e1000451 (2010).

MOLECULAR BIOLOGY

Getting Involved Locally

MicroRNAs (miRNAs) are small RNAs of around 22 nucleotides that bind to protein-coding messenger RNAs (mRNAs). They regulate function in diverse cellular processes, particularly during development and also in cancer. Understanding how miRNAs themselves are regulated is an emerging area of interest. The cyclin-dependent kinase inhibitor p27 negatively regulates the G₁ to S phase transition of the cell cycle. Translation of p27 mRNA is inhibited in many cancer cell types by two miRNAs, miR-221 and miR-222. However, p27 accumulates in nondividing (quiescent) cells, despite the presence of high levels of miR-221 and miR-222, suggesting an additional level of regulation. Kedde *et al.* have discovered that the ability of these miRNAs to inhibit p27 is regulated by another RNA binding protein, Pumilio-1. The binding site of Pumilio-1 in the 3' untranslated region of p27 is located close to the binding sites for miR-221 and miR-222. The authors found that Pumilio-1 binding could reconfigure the local RNA structure, thereby granting the two miRNAs access. They propose that entry into the cell cycle of quiescent cells involves phosphorylation of Pumilio 1, which stimulates its binding to p27 mRNA, thereby exposing the nearby miRNA binding sites. These results suggest that specific binding proteins can structurally remodel RNAs, like they do chromatin, in order to regulate function. — HP

Nat. Cell Biol. **12**, 10.1038/ncb2105 (2010).

ASTRONOMY

Milky, Not Dusty

As many of us now learn at a young age, our solar system hosts small planets close to the Sun and giant planets farther out. What do the first planetary systems that formed in our Galaxy look like? Sheehan *et al.* used the Spitzer Space Telescope to search for dust around stars that formed early in the Milky Way's history. The stars they observed are twice the age of our Sun. Detection of discs of dust and debris around these stars would be a sign that at least small planetary bodies formed around them, because these discs are generated and maintained through collisions between comets and asteroids. Of the total of 11 stars observed by the authors and 11 others observed

by other groups, none showed signs of dust. This does not necessarily imply that these stars do not host planetary systems; some of them are known to be orbited by giant planets. The lack of dust implies that some of these systems did not stop at planetesimal growth but proceeded to giant planet formation. They could be analogues of our solar system, where there is very little cometary dust left after the formation and evolution of the gas giant planets. — MJC

Mon. Not. R. Astron. Soc. 10.1111/j.1745-3933.2010.00936.x (2010).

APPLIED PHYSICS

Calling for a Quantum Hush

Coupling the mechanical and optical degrees of freedom of a system, in for example an atomic force microscope, provides the ability to measure displacements on exquisitely small length scales and to image surface height variations of just a single atom. As the systems are designed with better sensitivity (as required for the potential detection of gravity waves in large interferometers) noise can become an increasingly dominant issue. This noise can arise from back-action, whereby the radiation pressure caused by the probe light can perturb the motion of the mechanical system. Optomechanical systems are now entering a sensitivity regime where the light and mechanical motion can be described quantum-mechanically. In analogy to the



Too loud out there?

operation of noise reduction headphones, which record the ambient noise and then play it back with the amplitude negated to prompt destructive interference, Tsang and Caves propose a route to develop quantum noise cancellation for applications in optomechanical sensor systems. They break the problem down into a number of components and in a flowchart representation deal with each component separately, introducing an anti-noise path. In their admittedly idealized system they show how such an approach can deal with the back-action noise and should lead ultimately to improved sensors. — ISO

Phys. Rev. Lett. **105**, 123601 (2010).

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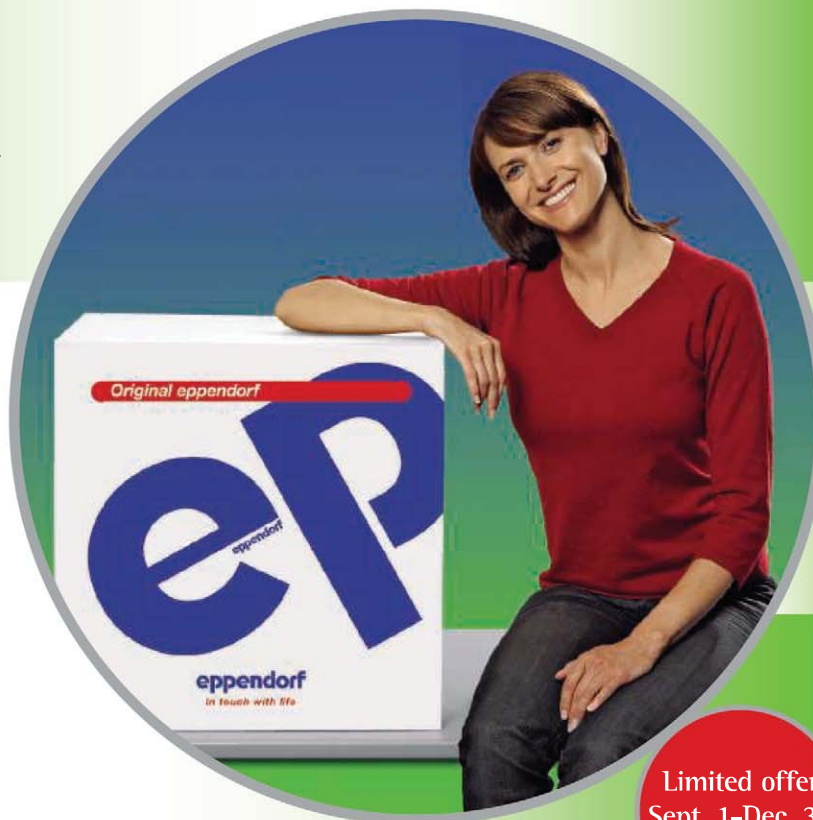
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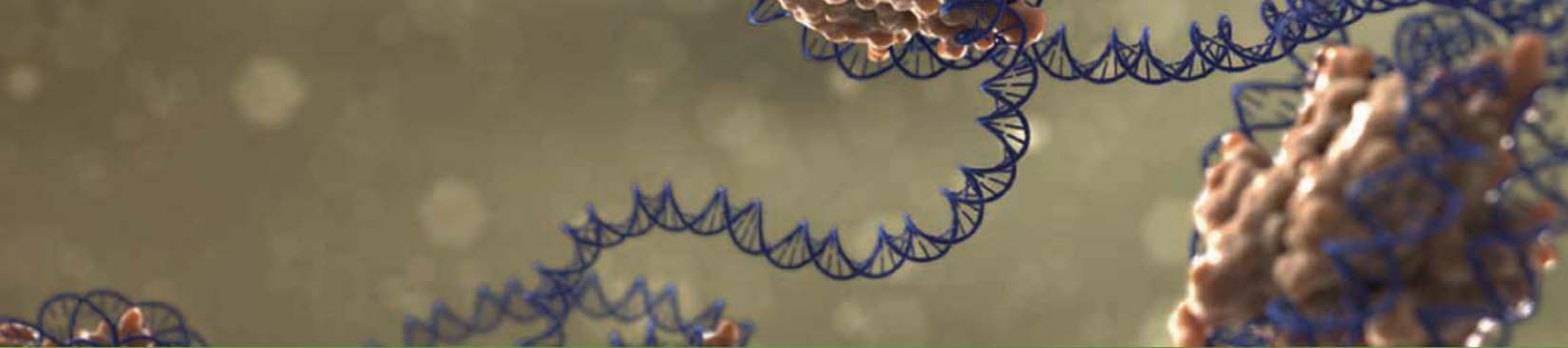
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SHELL GAME

It's a common belief among English gardeners: Throw the snails over the wall, and they'll come sliming back to eat your delphiniums another day. But common knowledge wasn't good enough for Ruth Brooks, a 69-year-old gardening enthusiast in Devon whose study of the phenomenon won BBC Radio 4's "So You Want to Be a Scientist?" amateur science contest in mid-September.

With guidance from David Hodgson, a population biologist at the University of Exeter whom the BBC assigned as her mentor, Brooks designed two experiments. First, she collected snails from two parts of her garden, marked them with nail polish, and set them down on a metal tray between the two spots. (When her family, she jokes, "saw me ... sitting there with my bright-red nail varnish marking snail shells, ... they did think I'd sort of flipped.") As garden lore predicted, most snails found their way back to their patch.

Then Brooks recruited people from around Britain to swap a bucket of marked snails from their own garden with a neighbor. Their reports showed that snails home reliably up to about 30 meters. So to be safe, Brooks recommends dropping them off more than 100 meters away.

Brooks says she was "delighted" to win, although it's debatable whether the prize, which came with no money, is a reward at all: She has to write up her results for publication in a journal.



Poop-Powered

The fuel of the future might be something we produce every day: excrement.

Last month at a park in Cambridge, Massachusetts, artist Matthew Mazzotta put the idea to the test with a methane digester fueled by



dog dung. Passersby picked up after their pets using biodegradable bags and threw the waste into an oxygen-free tank. As the microbes inside the dung broke it down, they released methane, which then zipped out through a tiny pipe to light up a gas streetlamp.

Machines like this aren't limited to animal droppings. "Anything that's organic you can use in these biodigesters," says Ahmad Pourmovahed,

a mechanical engineer at Kettering University in Flint, Michigan, who oversaw a much larger project to turn human waste into biogas. The real question is whether waste can compete against natural gas, a fuel whose price fluctuates constantly, he says.

The microbes don't do so well in cold weather, so on 25 September, Mazzotta had to power down the digester until spring. In the meantime, he hopes locals will think of a more creative way to use the fuel: to light a shadow play, say, or to brew tea at an eco-friendly teahouse.

Nobel Predictions

Can analyzing citations predict Nobel Prize winners?

As anticipation of the prize announcements (which begin on 4 October) mounted last week, Thomson Reuters released its annual list of "Citation Laureates," a roster of 21 potential Nobelists chosen based on citations, awards won, and the impact of their research. The recipe has predicted at least one Nobel winner in 19 of the past 21 years, although in some cases long before the year the prize was awarded.

Among those tipped in 2010: in chemistry, Patrick Brown of Stanford University for inventing the DNA microarray, and in physics, Thomas Ebbesen of the University of Strasbourg in France

for research in surface plasmon photonics. (See <http://science.thomsonreuters.com/nobel> for the full list.)

"I really do my best not to think about it," says Jeffrey Friedman of Rockefeller University in New York City, who, besides making the list in physiology or medicine, also won a Lasker Award this year (see below). Like most scientists, he prefers not to delve into Nobel hopefulness.

Lasker Awards

Breakthroughs in the biology of appetite and blood vessel growth and a lifetime of work on a blood disease have earned four scientists this year's Lasker Awards, considered the most prestigious prizes for biomedical research in the United States. Since the Laskers were first given out 65 years ago, 79 winners have gone on to collect a Nobel Prize.

Douglas Coleman, who is retired from the Jackson Laboratory in Bar Harbor, Maine, and Jeffrey Friedman of Rockefeller University in New York City share the basic research award for discovering the hormone leptin, which helps control appetite.

Napoleone Ferrara of Genentech in South San Francisco, California, wins the clinical award for his discovery of vascular endothelial growth factor (VEGF), a protein critical to blood vessel growth. Genentech has since developed two drugs that target VEGF, one for macular degeneration and the other for cancer.

A third award, recognizing special achievement, goes to David Weatherall of the University of Oxford in the United Kingdom, for his decades of work on the inherited blood disease thalassemia. Each award comes with \$250,000.



Coleman



Ferrara



Friedman



Weatherall

Dispute over a
new killer virus

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A vision for
Indian science

23



U.S. GRADUATE EDUCATION

Academy Rankings Tell You a Lot, But Not Who's No. 1 in Any Field

Perhaps it should be called the Mr. Potato Head of graduate school rankings.

Remember how easy it was to alter the appearance of that toy's bland, tubular face by sticking an ear or an eye in an unexpected place? Well, the latest analysis of the quality of U.S. research doctoral programs by the National Academies' National Research Council (NRC) can be manipulated in much the same way. But the exercise is hardly child's play.

This week's release of the long-awaited assessment, the first since 1995 and 3 years behind schedule, disgorges a massive amount of information about 5100 doctoral programs in 62 fields at 212 U.S. universities. More than a decade in the making, the assessment is meant to reflect the collective wisdom of the U.S. research community on what defines a top-quality graduate program. In an era of increased accountability, it's also designed to address questions from students, faculty members, university administrators, elected officials, and the public about the quality of any particular graduate program.

Yet that strength—the ability to serve as many audiences as possible—may also be

the assessment's most controversial feature. Those who simply want to know who's No. 1 in neuroscience, for example, or read a list of the top 10 graduate programs in any particular field will walk away disappointed after massaging the report's Excel spreadsheets, available at www.PhDs.org or www.nap.edu/rdp. That's because, like Mr. Potato Head, the NRC assessment can look quite different depending on your definition of "best."

To be sure, NRC does rank programs—but oh so carefully. Instead of assigning a single score to each program in a particular field, the assessment ranks the program on five different scales. Each score is also presented as a range of rankings reflecting the 5th and 95th percentiles of the scores it received. The scales themselves are based on 20 characteristics (see table, p. 19) that the NRC panel deemed appropriate for a quantitative assessment. Two are supposed to portray the overall quality of the program—one derived from a reputational survey (the R scale), the other from a quantitative analysis (the S scale). Three others rely on subsets that address important dimensions of quality: research activity, student support and

Number one? Duke was crowned the best men's college basketball team after winning the 2010 tournament, but where does its graduate program in chemistry rank? The new assessment of U.S. doctoral programs deliberately avoids a definitive answer.

outcomes, and diversity. The report itself highlights the uncertainties generated by such an exercise by calling the results "illustrative rankings [that] are neither endorsed nor recommended by the NRC as an authoritative conclusion about the relative quality of doctoral programs."

Given all those caveats, some university administrators are taking the rankings with more than a grain of salt. "We're pleased with how well our own programs ranked," says Patricia Gumport, dean of the graduate school at Stanford University. "But we have concerns about the methodology. So we're not planning to use the range of rankings."

It's easy to see the source of Gumport's concern by looking at what the assessment says about Stanford's anthropology department, to pick just one example. The department is ranked between 13th and 47th on the R scale and between 3rd and 9th on the S scale. In addition, it falls between 3rd and 14th on research activity, between 1st and 43rd on student support and outcomes, and between 12th and 33rd on diversity.

"It's difficult to draw meaningful conclusions about the relative quality of programs from these ranges of rankings," says Gumport with impressive understatement. Instead, she and her deans plan to mine the database to compare the performance of the university's 47 programs on one or more characteristics, or to see how a particular Stanford program stacks up with its peers around the country on those characteristics.

That's exactly what NRC hopes will happen. "We wanted to give people the chance to create rankings based on variables that they thought were important," says Charlotte Kuh, the NRC staffer who has lived and breathed the \$6.7 million assessment since it was launched in 2004 and who has made countless presentations to the graduate school community since then on its progress or lack thereof. That's especially true for different audiences, she adds: "While faculty have certain values, students may be worried about other things." For example,

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How to look for
life on Mars

26



Beyond ancient
DNA

28

an undergraduate who's thinking of becoming a microbiologist can find out how long it takes students at University X to complete their Ph.D. degrees, or what share of graduates from University Y find academic jobs. Likewise, an engineering dean interested in increasing the number of women or minority faculty members and students can compare the gender and racial diversity of her programs with those of others.

The absence of a single score separates NRC's rankings from those done by several other organizations, in particular, *U.S. News and World Report*, whose influential annual assessments of the "best" universities emulate those for college sports teams by offering the type of ordinal ranking that readers seem to crave. "We felt it was more responsible to be accurate," say Jeremiah Ostriker, a professor of astrophysics and former provost at Princeton University who chaired the committee that carried out the assessment. "That's especially the case if a small change in a range of rankings could make one school seem better than another."

To understand why one number is never enough, you need to understand how the panel went about its business. Asked by the National Academies to prepare a data-based assessment, the committee first whipped up a batch of 20 program characteristics. Then it served up those characteristics in two different ways.

The first involved asking 87,000 faculty members to weigh the importance of each characteristic. Then it applied those weights, in combination with data on those measures from institutions, faculty members, and students, to rate each one of 4838 programs in 59 fields. (It collected data but did not rate three fields that fell below a minimum size and frequency.) That's the S, or quantitative, ranking.

The second method asked 8000 faculty members to rank the overall quality of up to 15 programs in their field. Then NRC used a regression analysis to determine the perceived weights that each faculty member used in rating each program. It did this 500 times, selecting a different set of raters each time. That's the R, or reputational, ranking.

That process differs markedly from the 1995 assessment, which ranked programs based directly on their reputations. "This time around, we were not interested in the

WHAT COUNTS THE MOST?

Publications per allocated faculty member
Cites per publication
% of faculty members with grants
% of interdisciplinary faculty members
% non-Asian minority faculty members
% female faculty members
Awards/honors per allocated faculty members
Average GRE-Q score
% first-year students with full support
% first-year students with portable fellowships
% non-Asian minority students
% female students
% international students
Average number of Ph.D.s, 2002–06
% completing degree in 6 years
Time to degree
% students in academic positions
Student work space
Health insurance
Student activities offered

Pieces of the whole. The NRC report asked faculty members to weigh the relative importance of these 20 characteristics in determining a quality graduate program.



reputations per se," explains Ostriker. "Reputations suffer from many flaws, including a halo effect, time lag, and so on."

The panel intended to combine the R and S rankings into a single ranking, "for simplicity's sake," he says. But a National Academies' review panel blew the whistle on that approach. "They said we were actually measuring slightly different things and that they should be kept separate," explains Kuh. Ostriker says that readers can combine them by following a formula given in the appendix of a second volume, on the panel's methodology. "It's more confusing, but logically, I have to agree with the reviewers," he says.

The committee also took the review panel's advice to broaden the confidence level of each ranking from 50% to 90% (that is, to say there is a 90% chance that the quality of a given program falls within a particular range of rankings). At a 50% confidence level, the two rankings for a given program by definition overlapped only half the

time, an outcome that Ostriker says reviewers found confusing. On the other hand, as Gumpert points out, divergent scores like those for Stanford's anthropology program cast additional doubt on the NRC exercise.

One curious finding in this year's assessment is the correlation between a program's quality and its size. Faculty members did not put that characteristic high on their list, says Kuh. But, as in 1995, it shows up strongly in the rankings themselves. "What is it that size does?" Kuh asks. "I think it makes the program more visible. It's more likely to place students in other programs, and people from that institution have more articles in the literature, which may make readers think, 'Say, this must be a pretty good program.'" But the data show there's also a downside to size, she adds, with students from larger programs taking a longer time to earn their degrees.

The age of the data has attracted much criticism. Many university officials say the data—collected during the 2005–06 academic year—no longer present an accurate picture of many programs. "We've had 462 new faculty hires and 325 departures in the past 3 years," Gumpert notes, a significant proportion of the 1900-member faculty.

But the panel disagrees. Ostriker notes that the rankings are based in part on 5-year averages and says he doubts moving the time frame forward by a few years would have made much of a difference. In addition, the NRC questionnaire found that 80% of the faculty members had worked at their programs for at least 8 years. "That points to a huge level of stability," says Kuh.

For all the data's richness, however, panel members say they should not be used to determine the fate of a particular program. "None of the results dictates any decision, but they should stimulate discussion on why you are where you are," says Richard Wheeler, vice provost at the University of Illinois, Urbana-Champaign.

NRC hopes to update the database if it can raise sufficient funds to continue the project. In the meantime, says Ostriker, universities are encouraged to collect and disseminate new information about their graduate programs.

—JEFFREY MERVIS

INFECTIOUS DISEASES

Rival Teams Identify a Virus Behind Deaths in Central China

BEIJING—When Xue-jie Yu came to China last year to probe a lethal fever outbreak, everyone—Yu included—assumed he would provide damning testimony against a known suspect. Every summer for 3 years, hundreds of people in central China came down with an illness characterized by high fever and gastrointestinal (GI) distress. Many victims bled profusely, and an alarming number of the sick—rough estimates are as high as 30% in some areas—died. By early 2007, scientists at the Chinese Center for Disease Control and Prevention (CDC) here fingered the killer as human granulocytic anaplasmosis (HGA), an emerging bacterial infection from tick bites. But to Yu, an expert on tick-borne diseases at the University of Texas Medical Branch in Galveston, things didn't add up.

"The fatality rate was too high," Yu says, and in his experience it was "rare" for HGA patients to have GI symptoms. Working at the Chinese CDC's National Institute of Communicable Disease Control and Prevention (NICDC) here, Yu tested blood samples for *Anaplasma phagocytophilum*, the HGA bacterium—and came up empty. Last December, his team identified a new kind of bunyavirus, a family that includes infamous members such as hantavirus and Rift Valley fever virus. The finding, in a paper submitted to *The New England Journal of Medicine* (*NEJM*), unmasks a dangerous new emerging virus—not a bacterial outbreak—and explains why antibiotics failed to stop it.

Behind the scenes, however, a fierce argument has broken out over who discovered the

virus. This summer, a Chinese CDC team led by hantavirus expert Li Dexin, director of the agency's Institute of Virology, also uncovered a bunyavirus—possibly the same one Yu's group identified. They have submitted a deeper analysis of the pathogen, including complete RNA sequences of 11 strains, to *The Lancet*. Yu charges Li's group with trying to rob him of the discovery; Li says Yu's viral sequence is incomplete and that his team identified the virus as the killer.

Several key questions are disputed or unanswered. For starters, researchers do not know how lethal the virus is. The mortality rate may be high in China in part because clinics often prescribe the steroid dexamethasone to bring down high fevers; steroids suppress the immune system, which usually worsens infections. Scientists also differ on whether the virus should be handled in a biosafety level 3 facility—reserved for dangerous pathogens—or in less secure laboratories. And although the infection shows a seasonal pattern associated with tick-borne diseases—cases begin in early spring and peak in mid-summer before tapering off by autumn—the vector is still a mystery.

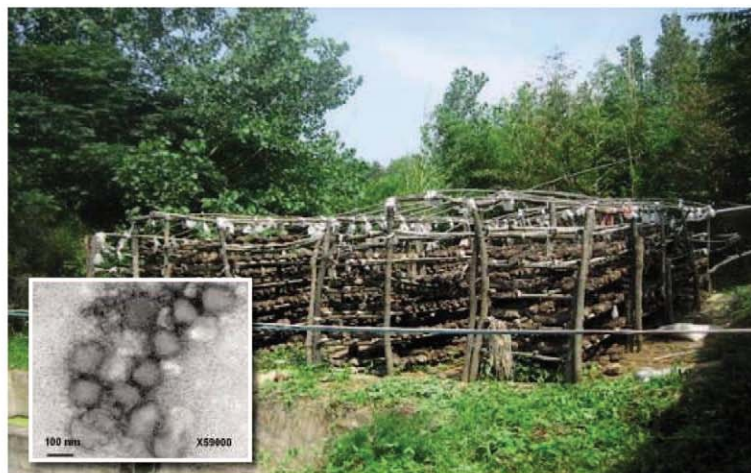
One indisputable fact is that the emerging disease has claimed scores of lives—mostly of farmers—in China's heartland. The first documented outbreak was in 2006, in Anhui Province. At that time, a team led by NICDC Director Xu Jianguo, Chinese CDC's chief bacteriologist, rushed to Anhui. Using PCR they found *A. phagocytophilum* DNA in the blood of one patient who died and in family members and hospital staff who became

infected. HGA had been recognized in the United States in 1990 and in Europe in 1997; Xu's group reported China's first cases in the 19 November 2008 issue of *The Journal of the American Medical Association*.

Curiously, however, none of the patients recalled having been bitten by ticks. And when outbreaks recurred in 2007 and 2008, the disease did not respond to antibiotics. Thinking it might have an unusual *Anaplasma* variant on its hands, NICDC in 2009 invited Yu as a short-term visiting researcher under the 1000 Talents Program, which brings overseas scientists to China.

That summer, the pathogen surfaced in Henan and Hubei provinces. In June, Yu went to Hubei's capital, Wuhan, to collect blood samples from patients. "They did not look like typical HGA cases," he says. After he failed to detect *A. phagocytophilum*, Yu says he urged Chinese CDC scientists to consider a viral pathogen—but researchers there flatly rejected the idea. Yu persisted and spotted virus particles that December in cell culture using electron microscopy. Then in February, he says, a member of his Texas lab, Yan Liu, "cracked the code of the viral genome." Two days after he informed scientists at the Chinese CDC about his findings, he says, his 1000 Talents affiliation with NICDC was terminated.

Chinese CDC Director Wang Yu was intrigued by Xue-jie Yu's findings and invited him to share them at a 15 April meeting at CDC headquarters to plot strategy for studying the disease. Among the attendees were Li and CDC virologist Liang Mifang; they found Yu's presentation unconvincing. "He said he isolated a bunyavirus, but he had gotten just fragments," says Liang. Yu confirms that the virologists were dismissive: "Li tried to deny the importance of my work," Yu says. Yu and his colleagues have named the virus Dabie



Into the hot zone. Xue-jie Yu (left, in blue shirt) looks on as farmers check a dog for ticks; forest-hugging farms (right) were hard hit by the emerging virus (inset).

CREDITS: COURTESY OF XUE-JIE YU/ UNIVERSITY OF TEXAS MEDICAL BRANCH; (INSET) COURTESY OF LI DEXIN/CHINESE CDC

Mountain virus after the range that straddles the borders of Hubei, Anhui, and Henan provinces where they collected samples. But Yu was not invited back to China this summer to continue his research. "I am the first scientist to discover the viral pathogen for an emerging infectious disease who has no access to study the virus and the disease anymore," he says.

In May, the Chinese CDC set up surveillance for the pathogen in Henan and Hubei provinces. The disease flared up in four other provinces as well, and Li's team collected blood and serum from all six affected provinces. They amplified viral RNA sequences and from more than 500 clones linked 14 to bunyavirus. They also isolated bunyavirus in

cell culture and sequenced 11 strains. They have named it severe febrile and thrombocytopenic syndrome (SFTS) virus and have classified it in the phlebovirus genus of bunyavirus. Li's group also detected the virus in 35 patients from three provinces. "It's solid work. They clearly show that a new virus is causing disease," says a U.S. scientist who has seen the data and asked to remain anonymous.

But the rival claim didn't sit well with Xue-jie Yu. When he heard that Li's team had submitted its findings to *The Lancet*, he sent an e-mail to the journal accusing Li of poaching his discovery. Liang says that's not true: "All data in our manuscript belong to us, not anyone else," she says. On 17 Septem-

ber, *The Lancet* asked Li's group to withdraw the paper and resubmit it after settling the authorship dispute.

The squabbling has put Wang, the Chinese CDC's director, in an awkward position. There is "no doubt," he says, that Xue-jie Yu "discovered the novel bunyavirus." While noting that Yu's results are not as "rich" as Li's team's, Wang says, "everyone knows what a scientific breakthrough is, and what is accumulating work." After the *NEJM* paper is published, he hopes, "other papers can go smoothly." But it may take Wang's best diplomatic skills to get any collaboration on the emerging virus to go smoothly.

—RICHARD STONE

ScienceNOW

From Science's Online Daily News Site



Strong as Silk

The silk produced by a Madagascar spider that spins webs as large as 2.8 square meters is the toughest biomaterial yet discovered, according to a new study in *PLoS ONE*.

Ingi Agnarsson, an entomologist at the University of Puerto Rico in San Juan, and colleagues collected Darwin's bark spiders (*Caerostris darwini*) from Madagascar's Andasibe-Mantadia National Park and brought them to a nearby greenhouse where the spiders could spin fresh webs. When the researchers compared Darwin's bark spiders' silk with other spiders' silk, they found that it ranked near the top in terms of strength and was twice as elastic as any other known spider silk, making it the toughest known biological material. Understanding the properties of spider silk could help engineers synthesize even tougher, lighter-weight materials, the researchers say. <http://scim.ag/strong-silk>

Primordial Magnetic Field

Two physicists say they have found an extremely weak magnetic field stretching across the universe, a possible remnant of the big bang. If scientists confirm the finding, it could help reveal the origins of magnetism in the cosmos.

Physicists Shin'ichiro Ando of the California Institute of Technology in Pasadena and Alexander Kusenko of the University of California, Los Angeles, knew that a vast magnetic field could scatter high-energy photons emitted by distant supermassive black holes, blurring the resulting telescope images. The effect would be too minuscule to see in a single image, so the pair created a composite image from data on 170 different black holes collected by the Fermi Gamma-ray Space Telescope. When they compared the composite with the product of a mathematical model that assumed no such field exists, the two images didn't match.

Their calculations, published in *The Astro-*

physical Journal Letters, suggest that the magnetic field responsible for the discrepancy has about one-quadrillionth the strength of Earth's. Although the researchers speculate that the field is a vestige of the big bang, others caution that it will require additional modeling to determine its source. <http://scim.ag/mag-field>

Viking Village

A team of archaeologists announced last week the discovery of a Viking settlement near the village of Annagassan, 70 kilometers north of Dublin. It could be the long-lost town of Linn Duhaill, one of two Irish outposts described in medieval accounts. The other, Dúbh Linn, became Dublin.

Annagassan filmmaker Ruth Cassidy and archaeological consultant Mark Clinton began searching for the town in 2005 and had almost given up when they came across an area ideal for building and repairing ships. A survey turned up a series of defensive ditches not typical of Irish construction, convincing the local

Louth County Museum to fund an excavation.

In just 3 weeks, the team found 200 objects, including evidence of carpentry, smelting, and ship repair, and hacked coins, which Clinton says were a typical "calling card" of the Vikings.



Other Viking experts are cautiously optimistic that the settlement is Linn Duhaill but say it needs to be solidly dated before the case is closed. <http://scim.ag/lost-village>

Read the full postings, comments, and more at <http://news.sciencemag.org/sciencenow>.



PSYCHOLOGY

Social Savvy Boosts the Collective Intelligence of Groups

People who are good at solving one type of brainteaser tend to excel at a variety of mental calisthenics—support, many psychologists say, for the concept of general intelligence. A study published online this week in *Science* (www.sciencemag.org/cgi/content/abstract/science.1193147) extends this concept to groups of people, arguing that groups have a “collective intelligence” that predicts their performance on a range of collaborative tasks.

The researchers, led by Anita Woolley, an organizational psychologist at Carnegie Mellon University in Pittsburgh, Pennsylvania, reached this conclusion after studying 699 people working in small groups. They also investigated why some groups appear to be smarter than others. Surprisingly, the average intelligence of the individuals in the group was not the best predictor of a group’s performance. The degree to which group members were attuned to social cues and their willingness to take turns speaking were more important, as was the proportion of women in the group.

“This paper really takes all the lessons of 100 years of psychometric research on individual intelligence and applies it in a novel way to look at group decision-making,” says Richard Haier, a neuroscientist at the University of California, Irvine, who studies intelligence. “You can get a lot of interesting ideas out of this.”

In the first part of the study, Woolley and colleagues at the Massachusetts Institute of Technology recruited 120 people from the Boston area and randomly assigned them to teams of three. Whereas most previous research has focused on what makes certain teams excel at a

given type of task, Woolley says she wanted to look instead at whether a team’s performance on one task generalizes to others.

Teams worked on a variety of tasks, including brainstorming to come up with possible uses for a brick and working collaboratively on problems from a test of general intelligence called Raven’s Advanced Progressive Matrices. These problems involve evaluating several shapes arranged in a grid and identifying the missing item that would complete the pattern. The groups also worked on more real-world scenarios, such as planning a shopping trip for a group of people who shared a car. The researchers scored these tests according to predetermined rules that considered several factors (awarding points when shoppers got to buy items on their list, for example). Each participant also took an abbreviated version of the Raven’s test as a measure of individual intelligence.

These experiments showed that a group’s performance on any one task did in fact predict its performance on the others. That suggests that groups have a consistent collective intelligence, Woolley says. She and colleagues calculated a “*c* factor” for each group, based on its performance across tasks, a direct parallel to the much-debated general intelligence factor, *g*. Neither the average intelligence of group members nor the intelligence of its smartest member correlated with the group’s performance.

To investigate further, Woolley and colleagues recruited another 579 people from Boston and Pittsburgh and assigned them to groups of two to five members. This time the researchers did find a weak correlation between both the average and the high-

est individual intelligence of members of a group and its collective intelligence. But other factors were stronger predictors. One was the group members’ average score on a test that required them to infer what was on another person’s mind—whether they were annoyed or worried, for instance—by looking at a photograph cropped to show just the eyes. That suggests that “social sensitivity” is a key ingredient of successful teams, Woolley says. The researchers found that the degree to which members took turns speaking also predicted their performance. The proportion of women in a group also correlated with collective intelligence, but Woolley says much of this effect can be explained by the gender difference in social sensitivity: women tend to have more of it.

The “careful, empirical experiments” are a welcome addition to the literature on teams, which is dominated by observational studies, says Brian Uzzi, a sociologist at Northwestern University in Evanston, Illinois. He agrees with the authors’ conclusion that the collective intelligence of groups may be more amenable to improvement than general intelligence in individuals, which most research suggests is difficult to change. Coaching to improve social perceptiveness and turn taking, or selecting individuals with those tendencies, might make for smarter groups, for example.

Research on how the gender composition of teams affects their performance has a long and controversial history, says Katherine Phillips, an organizational behaviorist at Northwestern. Some studies have found that women improve teams by virtue of their social acuity, whereas others have found that women are more likely to remain quiet and let others have their say in team discussions, sometimes to the detriment of the team. In the current experiments, women may have been more likely to speak up because none of the group members had particular expertise in the problems at hand, Phillips says.

However, the random makeup of the groups may limit the reach of the findings, cautions Linda Gottfredson, a sociologist who studies intelligence at the University of Delaware, Newark. She notes that the groups were composed of strangers. “It is possible that turn taking in conversation was so important for that reason,” she says. “They did not know how bright and sensible the others were.” In a more typical workplace setting, Gottfredson says, individuals would be more familiar with their teammates and know whom to listen to and encourage to speak. In that case, she says, the members’ individual intelligence may be a more important factor than turn taking.

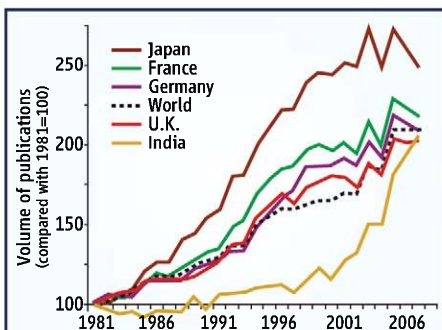
—GREG MILLER

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SCIENCE POLICY

India's Vision: From Scientific Pipsqueak to Powerhouse

NEW DELHI—In 1930, Indian physicist C. V. Raman won a Nobel Prize for his discovery of inelastic photon scattering, known as the Raman effect. The phenomenon became a powerful tool for analyzing matter—but it was other countries that used the basic knowledge to invent Raman scanners. That still causes heartburn here. In a new report, a blue-ribbon panel cites Raman as one egregious example of India's systemic failure to capitalize on basic research findings.



False positive? Although India is grabbing a larger share of scientific publications, a panel led by chemist C. N. R. Rao argues that India's scientific stature is shrinking.

The report, released last week by Prime Minister Manmohan Singh, offers a stinging indictment of India's scientific frailties, noting that science here is "severely hampered by oppressive bureaucratic practices and inflexible administrative and financial controls." Titled *India as a Global Leader in Science*, the "vision document" also offers a blueprint for strengthening Indian science—one that will require heaps of money to implement.

"We would need to redouble our efforts and hope that the ideas in the vision document will inspire the scientific community," Singh said in releasing the report. The first of its kind, the report is getting mixed reviews. Goverdhan Mehta, an organic chemist at the University of Hyderabad and past president

of the International Council for Science in Paris, says the recommendations are sound. "If India is to become a formidable force, incremental approaches will just not work. One needs to leapfrog," Mehta says. Others are unimpressed. It is a "very environment-friendly document since it has recycled so many old ideas," scoffs one senior scientist.

Commissioned by Singh's office, the panel, led by the chair of the prime minister's scientific advisory council, C. N. R. Rao, a chemist at Jawaharlal Nehru Center for Advanced Scientific Research in Bangalore, flatly declares in its report that "India is yet to become a major force in global science. ... Indeed India's *relative* position in the world of science has declined in the last twenty years." It blames inadequate investment in science both by the government and by industry. That has led to a disconnect between basic research labs and industry, says chemical engineer Raghunath A. Mashelkar, now president of the Global Research Alliance in Pretoria. The paradigm of "science being born in India but products being born overseas has to be overturned," he says.

"To begin to contribute significantly to world science," the report says, India's share of scientific papers should rise from the present 2% or so to "something like 10%" over the next 10 years. It also urges Indian researchers to claim more intellectual property: The panel calls for a 10-fold increase in international patents owned by Indians, from 1900 in 2007 to about 20,000 by 2020. And training scientists should get a major boost: India should produce about 30,000 science Ph.D.s a year by 2025, up from 8420 in 2006. To meet those targets, the government should double science spending by 2020, the panel says. It also seeks a \$250-million-a-year venture capital fund to develop basic research findings. And the panel calls for the creation of a National S&T Council along the lines of the U.S. National Research Council to help India address urgent issues such as water, energy, and food security.

"We will seriously try to implement the vision," India's Science Minister Prithviraj Chavan told *Science*. Any new funding initiatives would be considered in the 2011 budget. Rao is not optimistic. "I feel a bit depressed and discouraged by the state of Indian science," he says.

—PALLAVA BAGLA

Science Insider

From the *Science* Policy Blog



Government lawyers asked an appeals court to suspend a federal judge's ruling that froze federally funded research on **human embryonic stem cells**. The three-judge panel had tough questions for both sides, but at press time, the court had not yet ruled on whether to allow research to continue while the case proceeds. http://scim.ag/stem_hearing

Weeks after it emerged that four papers written by **prominent gene therapy expert Savio Woo** had been retracted and two postdocs subsequently fired, two more papers have been retracted. Mount Sinai School of Medicine in New York City has cleared Woo of wrongdoing, and several papers on his techniques have not been retracted. http://scim.ag/New_Woo_papers

The outgoing **chair of the science committee** in the U.S. House of Representatives says he wishes he had pushed harder to pass the reauthorization of the America COMPETES Act, which supports increased U.S. spending on research and science education. But Representative Bart Gordon (D-TN) still hopes the Senate will take up the House-passed version in the lame-duck session after next month's elections. Meanwhile, top U.K. science officials have lobbied the British government to avert **spending cuts** expected to be part of next year's budget, to be released 20 October. http://scim.ag/competes_late
<http://scim.ag/UKcuts>

Chinese police have detained a top surgeon in an **investigation into the assaults** on a critic of medical research and an investigative journalist. Xiao Chuanguo, chief urology surgeon at Tongji Hospital in Wuhan, was detained in connection with attacks on misconduct watchdog Fang Shimin and reporter Fang Xuanchang. <http://scim.ag/XiaoDetained>

Scientists can entice the **public to donate to their work** on a new philanthropy site called SciFlies. Started by a veteran political fundraiser and scientist with experience as an entrepreneur, the site hopes to raise funds for grants as large as \$100,000 to support scientists in all fields. http://scim.ag/sci_donate

For more science policy news, visit <http://news.sciencemag.org/scienceinsider>.

CONSERVATION

Filling Gaps in Global Biodiversity Estimates

Data deficient. Biologists use this term to describe a species for which too little is known to determine its conservation status. It's a problem at a global scale as well, as scientists try to assess whether biodiversity loss has been stemmed since the Convention on Biological Diversity (CBD) was enacted almost 20 years ago. Scientists, who hope to get new conservation targets approved later this month (*Science*, 10 September, p. 1272), have had comprehensive information on just a few groups of organisms, such as birds and mammals, but not on some important large groups—

Over the past 2 years, other research teams have been applying this approach to poorly understood groups of animals. For both plants and animals, researchers plan to look at these same sets of species periodically to see how the groups are faring and thus how well humans are protecting biodiversity.

The new index is a response to growing frustrations with the International Union for Conservation of Nature's (IUCN's) Red List of Threatened Species, which is an ongoing tally of which species are known to be in trouble. Its data are used to tell how well the CBD is working. The Red List now contains entries on about 45,000 species; birds, mammals, amphibians, and corals are especially well-documented.

Those groups are relatively easy to track because each contains a small number of species (say, several thousand) and many experts are studying them—especially birds and mammals. The world's 10,000 avian species have been sampled six times since the early 1970s, for instance, providing enough information to conclude that birds are slightly worse off now than they were then. But for many of the rest of the biosphere's 1.75 million known plants, fungi, and animals, the ratio of species to experts is much less favorable. Who can do a detailed analysis of the range, distribution, and ecology of each of the 380,000 estimated plant species, for example, or 75,000 butterflies, just once, let alone periodically to figure out the trends?

In 2005, IUCN recruited experts to see whether a statistical sampling technique, applied to a particular group of flora or fauna, could fill the void—and if so, just how small the sample could be. “It's like a poll; you want to know with which degree of certainty you can determine trends through time,” says Jonathan Baillie, a zoologist at the Zoological Society of London who helped develop this index.

A sampled approach would work only if experts could draw on a comprehensive list of species in that group. Using plants and birds as test cases, statisticians decided they needed a minimum sample size of 1500. That was large enough so that the protocol would work even if 40% of the randomly selected species were busts—with no conservation data available.

Kew took on plants, finishing the new analysis just in time for this month's U.N. Bio-

diversity Summit. Working with Kew geographic information system specialist Steve Bachman and volunteers, Nic Lughadha and her collaborators looked at ferns and their relatives, conifers and cycads, and also the monocots, the 75,000-strong group of flowering plants that includes grasses, palms, and lilies. They did not have a comprehensive list for the remainder of flowering plants, formerly known as the dicots, but determined they could study 1500 legumes—a major dicot subgroup—as stand-ins.

For some of these randomly picked species, the researchers had little to go on, so there was a “mad scramble” to track down any information about their distribution and ranges, says Neil Brummitt, now with the Natural History Museum in London. Often, they had to rely on labels on herbarium specimens to get a sense of how a plant's range and distribution, for instance, changed through time.

Other groups' estimates of the risk of extinction were based primarily on studies of a small group of plants or plants in a particular geographic location, says Nic Lughadha, who adds that the new index provides the first global picture of how plants are doing: in short, about the same as mammals, but worse than birds.

The new technique has already provided insights into the world's fauna. Freshwater crabs and fish, two data-deficient groups, are at higher risk than their marine counterparts. And some trends are emerging—such as the potential advantage of having wings. Researchers already knew that birds are better off than mammals and amphibians; similarly, the newly examined dragonflies are in less danger than crayfish and crabs. “It really has improved our knowledge of some of these groups immensely,” says IUCN's Craig Hilton-Taylor.

The index approach has its limitations. “By not doing the whole group, you have less information to direct conservation priorities” at the local level, says Hilton-Taylor. And because selection is random, some highly vulnerable species may be left out, says Sacha Spector, an ecologist with the environmental organization Scenic Hudson in Poughkeepsie, New York. Even so, he thinks the sampled approach is providing a broad picture of what's happening: “It will be extremely successful in jump-starting the biological research world [for] doing more assessments and protecting the species that were under the radar before.”

—ELIZABETH PENNISI



Taking stock. By sampling various groups, including (clockwise from left) lycophytes, snowdrops, and cycads, researchers have determined the conservation status of plants as a whole.

especially plants. Because they are so critical to the survival of other species, “we think plants may be more representative of what's happening to the whole of biodiversity,” says Eimear Nic Lughadha of the Royal Botanic Gardens, Kew, in the United Kingdom.

Earlier this week, researchers led by Nic Lughadha helped fill this data gap by using an innovative statistical approach called the Sampled Red List Index. By taking a random sample of the known species in various plant groups, such as ferns, and compiling existing information on just that subset, they have concluded that 22% of all plant species are threatened. Previous estimates had ranged between 20% and 80%, making it difficult for policymakers to know how much they should emphasize plants in their conservation strategies.

CREDITS: (BOTTOM LEFT) MARTIN CHEEK, RBG KEW; (TOP AND RIGHT) RBG KEW

NEWSMAKER INTERVIEW: IAN POINER

Counting the Ocean's Creatures, Great and Small

In a massive undertaking, 2700 scientists from 80 nations have spent the past 10 years compiling everything known about life in the world's oceans: from extinct denizens to present-day biota to what future ecosystems might look like. Next week, participants will gather in London to celebrate the fruits of their labors: the first ever Census of Marine Life (CoML).

Launched in 2000 with funding from the Alfred P. Sloan Foundation, CoML gathered some 16 million records into accessible databases. Fieldwork by CoML contributors has added another 2600 publications, including 6000 or so new species, raising the number of known marine species to over 240,000—about 25% of the total presumed to exist.

The \$670 million effort “has been good value for dollar spent,” says Ian Poiner, chief executive of the Australian Institute of Marine Science in Townsville. The tropical marine ecologist was involved in the census from the beginning and has chaired the scientific steering committee since 2008. Although CoML is a milestone, many questions about ocean life remain unanswered, Poiner notes: “The age of discovery is still with us.”

—DENNIS NORMILE

Q: Has CoML achieved its goals?

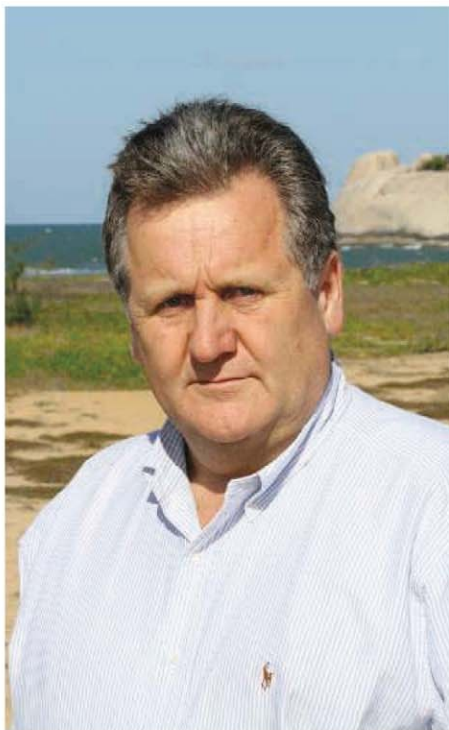
I.P.: The census has three key points of focus: One is diversity, or what lives in the ocean; the distribution, where that life is found; and then the abundance, how much life is there. On top of that we looked at what changes have occurred and forecast what [ocean life] might look like in the future.

I think we dealt with diversity very well; distribution and abundance are much more difficult. There is still much to be done, particularly on abundance.

Q: Do you have an idea of what kinds of species are still out there?

I.P.: When you plot the accumulated number of species over time, it's still going upward with no hint of leveling out. Census researchers estimate there will be at least a million species and possibly many more. We know less about the smaller things than the bigger things. And marine microbes are another story. Probably 90% of the ocean biomass will be microbes.

Q: Why is it important to identify those species?



“The census found life everywhere we looked, and it is much more complex and interconnected than we expected.”

—IAN POINER

I.P.: Most ecologists view biodiversity as a measure of the health of biological systems. What are all the species doing out there and what's their role in ocean ecosystems? Those questions remain to be sorted out. Getting a better handle on biodiversity and understanding [the implications] of declines in biodiversity and species invasions are things we need to know to effectively manage our oceans for both conservation and for economic benefit.

Q: Aside from the sheer numbers, what are the most significant findings?

I.P.: Things like discovering animals that live without oxygen in the deep Mediterranean. On the Great Barrier Reef, probably a third to a half of the soft corals are new to science. One of the iconic species of the census is the yeti crab from the Pacific near Easter Island. It is not only a new species but a new genus and a new family. There are

many of those sorts of discoveries.

The census found life everywhere we looked, and it is much more complex and interconnected than we expected. Probably the other [key finding] is that we humans have had far more impact on the oceans than we had imagined.

Q: The CoML is regularly mentioned in the comic strip *Sherman's Lagoon*. Was that part of your public outreach efforts?

I.P.: We encouraged and supported it. It's one way to communicate the importance of ocean life. But it is only one of many avenues we've used to enhance the general recognition of the importance of our oceans. *National Geographic* will also publish a two-sided wall map depicting the major findings of the census.

Q: Is census data being used to understand the impact of the Gulf of Mexico oil spill?

I.P.: The short answer is yes. We released last year the first comprehensive compilation of the biodiversity of the Gulf of Mexico. Sadly, it was timely. It has been and will continue to be used to understand the impact of the Gulf of Mexico oil spill.

Q: Who else will use census data?

I.P.: Census data are already being used for the upcoming Convention on Biodiversity, both by countries reporting their ocean biodiversity and in looking at options for the management of our high seas. [This information] will be used much more extensively in the future. An example is the Great Barrier Reef; it is a large area and one of the most iconic places in the world. The management and zoning and systems to maintain protected areas and multiple-use areas are dependent on the information that has been collected in the census.

Q: What comes next?

I.P.: The census was very much a bottom-up process where the marine science community came together to identify challenges and identify ways to collaborate. Now we are using those networks to help us determine where to go in the future.

This census is the first of, hopefully, many. With the technologies [CoML has] developed, we're now in a much stronger position to continue discovery and exploration and to monitor changes in ocean biological patterns. Challenges [include] getting the balance between discovery and monitoring right and demonstrating the effective use of that information in assessing and managing our oceans.

Growing Prospects For Life on Mars Divide Astrobiologists

As discoveries on Mars, including warm spells and salty soil, raise the chances of finding life there, scientists consider how best to look for it within their budget

Looking good. Phoenix found water ice and benign living conditions in the martian Arctic.

THE MICROSCOPIC, WORMY-LOOKING things found in a meteorite blasted off Mars certainly reinvigorated NASA's search for extraterrestrial life in the 1990s. But the critterlike sightings and almost all of the other evidence for ancient life claimed for the martian meteorite have since faded away. Replacing them in recent years is a string of encouraging discoveries on Mars, including pervasive ice just beneath polar soils, water-wrought minerals of every sort, and soil benign enough to grow asparagus.

"Mars has met all requirements to support life," says planetary scientist and astrobiologist Bruce Jakosky of the University of Colorado, Boulder. "Life is possible." But as the planetary science community draws up its prioritized list of missions for the next decade, astrobiologists have split over the next logical step in the search for life on Mars.

Many researchers are content to follow NASA's lead as it cautiously moves from "following the water" in search of likely habitable or once-habitable environments to "following the carbon"—that is, looking for chemical traces of ancient life. Others are not so patient. "It's time to search for life by searching for life," says astrobiologist Carol Stoker of NASA's Ames Research Center (ARC) in Mountain View, California. The direct detection of life—living, dormant, or recently deceased—should be a high priority, she and others say. The planetary science

decadal survey now being drafted and to be released next March will determine whether they're right.

A life-friendlier planet

The search for martian life took a body blow in the late 1970s, when life-detection experiments on the Viking 1 and Viking 2 landers failed to stir up clear signs of either living creatures or lifeless organic debris. Mars seemed to be dead, as dead as an eons-old, hyperarid, incredibly cold landscape would appear to be.

It wasn't long, however, before things started looking up again. Orbital observations from Viking onward revealed ever more evidence that water gushed across the surface of early Mars, forming lakes and perhaps even

a northern ocean at about the time life was getting started on Earth. Spectra returned by orbiters in the past decade showed that liquid water on early Mars stayed around long enough to corrode rock into alteration minerals under life-friendly conditions. And the Mars rover Spirit found ancient minerals produced by hot ground waters, much as happens in the life-infested hot springs of Yellowstone.

Closer to modern times, water—albeit frozen—is turning up in all manner of places. In recent years, orbiting instruments have spied widespread ice just beneath the surface soils of Mars: centimeters down in polar regions and meters down in immobile glaciers at mid-latitudes. But that water might not always have been frozen. Mars's wobbling on its axis would have warmed parts of the planet. Orbitally induced climate cycles mean that Mars has become "a far more interesting place than we thought," says astrobiologist David Des Marais of ARC. The fresh-looking gullies of Mars, for example, may have been gushing meltwater in the not-so-distant geologic past.

With things looking up for biology on Mars, NASA sent the Phoenix lander to a landing site in the martian Arctic that turned out to be relatively life-friendly. "We believe we have a habitable environment in modern times," says Stoker, who is on the Phoenix team. The site is "not necessarily



Stymied. The Viking landers found no signs of life.

CREDITS (TOP TO BOTTOM): NASA/JPL-CALTECH/UNIVERSITY OF ARIZONA; NASA/JPL-CALTECH/UNIVERSITY OF ARIZONA; NASA/JPL-CALTECH/UNIVERSITY OF ARIZONA

growing organisms today,” she says, but “over the past 10 million years during warm conditions,” life could have survived and perhaps thrived.

To put a number on that assertion, Stoker and 12 co-authors published a “semiquantitative” analysis of the Phoenix site’s potential habitability online 16 June in the *Journal of Geophysical Research-Planets (JGR)*. They considered the likelihood of four factors essential for life. One is liquid water. Phoenix’s discovery of carbonate requires liquid water in the past to produce the mineral, they noted. The high concentration of perchlorate salt found by Phoenix offers a means of forming brines that could remain liquid even at today’s temperatures. And even without brines, orbitally induced warming could have allowed liquid water in the recent past.

Phoenix’s perchlorates could also serve as an essential energy source for microbes with a taste for such chemicals, the group said. Nutrients such as nitrogen and phosphorus blow in on dust. And environmental conditions, such as the Phoenix-measured pH, are benign. All that makes martian high latitudes “more tantalizing,” says atmospheric chemist Sushil Atreya of the University of Michigan, Ann Arbor.

A lively Mars, ever?

An even more tantalizing hint of possible current life showed up in 2003. Researchers reported finding traces of methane gas in the atmosphere of Mars, first in spectroscopic observations returned by Mars Express and then in telescopic observations from Earth. A lifeless, subsurface water-rock reaction called serpentinization could be the source, but living, methane-belching microbes would work, too.

Another encouraging sign just came from the laboratory. Astrobiologist Rafael Navarro-González of the National Autonomous University of Mexico in Mexico City and his colleagues report in a paper in press in *JGR* their terrestrial version of the Viking search for organic matter on Mars. In the Viking experiments, a martian soil sample was heated to 500°C and the resulting gases analyzed. Instead of organics from the soil, Viking detected only carbon dioxide and two chlorinated methane compounds. The latter were considered to be contaminants brought from Earth, even though they did not appear when the experiment was run without soil while Viking was in space.

Navarro-González and his colleagues, including Christopher McKay of ARC, reran the Viking experiments in the lab using the



Following the carbon. The Curiosity rover, due for launch next year, will minutely analyze any organic matter it finds.

most Mars-like soil available, one from the heart of the Atacama Desert of Chile containing a trace of organic matter. When they added a bit of perchlorate before the heating, in line with the Phoenix discovery, they duplicated the Viking results: no volatilized organics, some carbon dioxide, and the same two chlorinated methane compounds.

“The simplest explanation is that there was perchlorate and organic matter in the Viking soil samples,” says McKay. When heated, the perchlorate would have oxidized most of the organic matter to carbon dioxide and chlorinated a small amount of it. If the Viking soil did contain organic matter, it could have come from lifeless meteorites and cosmic dust that rains onto Mars. But “we did dispel the idea that there’s no point in searching for” molecular traces of life in martian organic matter, McKay says.

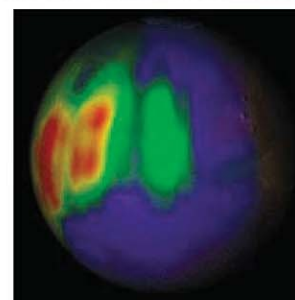
Go for it?

All the upbeat developments have some astrobiologists raring to go. “The theme has been ‘Follow the water,’ ” says Stoker, “but we understand enough to take the next step.” That step would be attempting to directly detect active or dormant present-day life. “The probability of identifying life is higher for modern” than for ancient life, says Stoker. The best approach, McKay says, is to send modern biochemical sensors to Mars capable of directly detecting complex biomolecules like DNA from living or dormant organisms. Such a prospect has made some astrobiologists “impatient to do something directly relevant to the search for life,” says McKay, “rather than taking ‘more pictures of rocks.’ ”

NASA’s astrobiology plans for Mars are “geared much more toward ancient life than present-day life,” notes Jakosky. In 2011, NASA will send the Mars Science Laboratory, now dubbed Curiosity, to follow the carbon at a low-latitude site. In a joint 2016 effort with the European Space Agency, it will send the Trace Gas Orbiter to check on the methane. And for 2018, NASA is consid-

ering a rover that would collect likely samples for eventual return to Earth and detailed laboratory analysis for ancient life.

A search for ancient life has plenty of supporters. “A majority [of astrobiologists] would say ancient life will be easier to find than present-day life,” says Jakosky. To find present-day life, researchers have to first identify a likely site, he says. Then they would have to choose which measurements would detect alien life. In hindsight, Viking scientists erred in designing their life-detection experiments for microbes accustomed to Earth’s most benign conditions. And ruling out microbial contamination from Earth would be challenging. Although going after present-day life could yield “the most profound result,” says Jakosky, it would be “very risky. The odds are against it even if life is present.”



Getting closer? Viking (left) found no life, but apparent recent water flows (top) and methane (above) hint at it.

A committee of the National Research Council is now balancing the odds of success of the two approaches against the available funding in the United States as it finishes the first draft of the Planetary Science Decadal Survey. Its prioritized list of missions for 2013 to 2022 is “exciting and actually implementable,” says committee chair Steven Squyres of Cornell University. How lively its missions to Mars will be remains to be seen.

—RICHARD A. KERR

CREDITS (TOP TO BOTTOM): THOMAS A. DUTCH/SLAGER/NASA/JPL/CALTECH; NASA/JPL/UNIVERSITY OF ARIZONA; NASA/JPL; NASA



ARCHAEOLOGY

Archaeologists See Big Promise In Going Molecular

Creative applications for ancient DNA emerge at a meeting, where researchers also described studies of other preserved molecules, including ancient RNA and proteins

COPENHAGEN—As a field, ancient DNA is getting, well, old. It's been more than 2 decades since scientists began analyzing fragments of preserved DNA from tissues that are hundreds, or even tens of thousands, of years old (*Science*, 17 November 2006, p. 1068); the approach arguably reached a climax earlier this year with the unveiling of the draft nuclear genomes of a 4000-year-old Eskimo and a 38,000-year-old Neandertal.

Yet last month's International Symposium on Biomolecular Archaeology (ISBA)—a biennial meeting devoted to studies of isotopes and DNA in ancient biological materials—revealed that ancient DNA research is poised to undergo a new kind of growth spurt. Flooded with data from next-generation sequencing technologies, researchers are now exploring a host of new applications and addressing an ever-wider circle of questions about ancient ecologies, human behavior, and lifestyle. They're probing ancient medical practices by extracting DNA from Roman herbs, for example.

Some are moving beyond isotopes and DNA to probe new classes of ancient molecules, using ancient RNA to study gene activity and identifying ancient species with bits of collagen from fossil bones. "Everyone is in a rush to find the next methodology," says bioarchaeologist Greger Larson of Durham University in the United Kingdom. "We are all testing things very quickly without knowing how successful any will be."

Seed of an idea

Take ancient RNA, for example. Few thought it made sense to even look for DNA's cousin in ancient biological samples, because RNA is much more fragile than DNA. But M. Thomas Gilbert of the University of Copenhagen's new Centre for Geogenetics (an ambitious ancient DNA lab funded by the Danish government and officially launched at the start of the meeting) became intrigued years ago when he read of a date palm germinating from a 2000-year-old seed from the ancient Jewish fortress of Masada. To Gilbert, that suggested the ancient seed still harbored RNA and that other old seeds might as well. At the meeting, Gilbert's colleague Sarah Fordyce reported that they have been able to sequence many RNAs, including the messenger RNAs (mRNAs) made when genes are transcribed, from Chilean and North American maize seeds that are more than 700 years old. "We think we can do ancient transcriptomics," she says.

Seeds are a good source for ancient RNA, because they have evolved to keep genetic material intact under harsh conditions. "Seeds are particularly valuable objects for studies of ancient nucleic acids," says Matti Leino of the Swedish Museum of Cultural History in Stockholm, whose own group recently sequenced DNA in historical seed collections and found evidence challenging the importance of a mutation hypothesized to be central to wheat domestication.

Preserved RNA in seeds could be a boon to studies of ancient plant domestication, as levels of mRNAs reflect changes in gene activity that may be as important to the process as changes in protein-coding DNA sequences. A plant's RNA profile differs from tissue to tissue, but Gilbert's team suspects that seeds, as a crucial developmental stage, nonetheless will offer key insights. Gilbert's is not the only team that has discovered the protective power of seeds; a group led by Robin Allaby of the University of Warwick in the United Kingdom has isolated RNAs from ancient Egyptian barley seeds and hopes to identify a variety of RNAs that regulate gene activity.

ZooMSing in on collagen

RNA may be considered fragile, but the bone protein collagen is among the best survivors of the ravages of time. RNA can last hundreds of years, and DNA tens of thousands, but collagen apparently sometimes survives for millions of years in a bone. That's why Michael

Buckley of Bournemouth University, while working in the laboratory of Matthew Collins of the University of York, both in the U.K., spearheaded a technique to use collagen fragments, or peptides, to identify the species of

nondescript bits of bone. Called ZooArchaeology by Mass Spectrometry (ZooMS), the method relies on subtle differences among species in the amino acid sequence, and thus mass, of collagen. For less than \$10 a test, scientists may one day be able to take any bit of fossilized bone and identify the genus, or even species, it came from, says Buckley. (Collins has nicknamed collagen the "bar code of death" because it can identify fossils, whereas DNA is sometimes used as a bar code to identify living species.)

Collins's team has so far demonstrated ZooMS's ability to identify a wide range of

Online

sciencemag.org

Podcast interview with author John Travis.

CREDITS (LEFT TO RIGHT): GIOVANNI LATTANZI/ARCHAET; MATTI LEINO/SWEDISH MUSEUM OF CULTURAL HISTORY



fossil animal bones. It can even distinguish between ancient sheep and goat bones, a notoriously difficult problem that has blurred the picture of how people domesticated each animal. Collins's group is now building up a database of collagen's amino acid sequences that more fully spans the animal kingdom. ZooMS is "fast and cheap and can be a real neat way to ID species," says Gilbert. And it has other potential uses, such as identifying the collagen sequence of extinct species for which no DNA has been isolated, helping better place those animals within an evolutionary tree.

"Every couple of weeks, we think of something new to do with it," says paleobiologist Ian Barnes of Royal Holloway, University of London, who has begun to collaborate with Collins's group.

Other researchers suspect that the future of ancient proteomics may involve more than just collagen, as there's increasing evidence that many proteins in bones and in other biological materials can survive for long periods, at least in a fragmented form that can be analyzed by mass spectroscopy. Collins's team is trying to extend the ZooMS technique to keratin proteins, to analyze animal hair and feathers used by ancient peoples, for example.

An ancient pharmacopeia

As the proteomics researchers looked to the future, ancient DNA researchers presented a few new tricks of their own. Botanist Alain Touwaide of the Smithsonian Institution in Washington, D.C., studies ancient "pharmacological" texts to understand how ancient people treated illnesses. But a sunken 1st century Roman shipwreck containing a host of medical items, including pill-like objects still dry in waterproof clay cylinders, offered him an unusual chance to verify those documents. Touwaide suspected that the pills or tablets, which may have been applied to skin rather than ingested, were

medicines composed of ground-up plants, so he recruited Smithsonian ancient DNA expert Robert Fleischer to analyze two tablets provided by an Italian museum.

At the meeting, Fleischer reported that he has indeed found chloroplast DNA in the tablets, so far identifying 10 different types of plants, including celery, carrots, and hibiscus. Touwaide hopes ultimately to match the tablets' contents to one of his texts and identify its possible therapeutic use. It would be the first time that "ancient medicines are analyzed and then interpreted on the basis of ancient scientific literature," he says. "The

New tricks for old DNA. Researchers are analyzing ancient DNA from (left to right) medicines in Roman containers, seed collections, Viking wool textiles, and eggshells of extinct birds.

Orlando of the Centre for Geogenetics says he likes "the idea of getting access to ancient pill recipes using DNA. This approach can be really powerful."

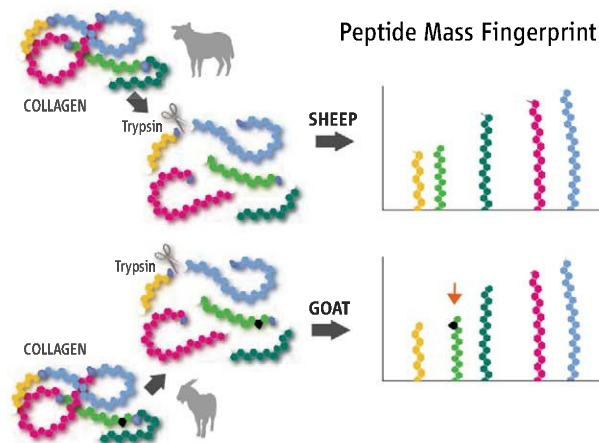
Other ISBA presentations extended ancient DNA studies to new types of preserved biological specimens. For example, Charlotte Oskam and Michael Bunce of Murdoch University in Perth, Australia, have developed

a method to tease out DNA sequences from fossil eggshells. They're using it to identify fragments smashed and burned shell fragments left by the hunters who colonized New Zealand about 700 years ago. These people apparently drove large flightless birds such as the moa to extinction, and identifying the species and quantity of eggs at each site may suggest how quickly this happened. And Luise Brandt in Gilbert's group reported the first success at obtaining DNA from ancient wool textiles; researchers had worried that wool processing destroyed genetic material.

The technique could reveal where the wool came from, illuminating early trade routes for textiles.

ISBA is a young conference, begun in only 2004, and to date it has always been held in Europe. But in 2012 it moves to China, whose representatives lobbied hard to host the meeting. For China, an ancient country with an increasing passion for cutting-edge science, biomolecular archaeology is apparently an irresistible combination.

—JOHN TRAVIS

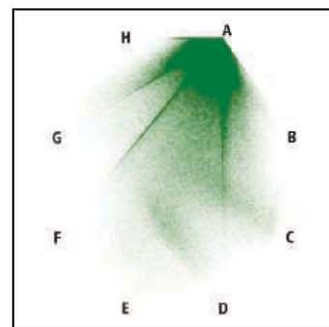


Separating the sheep from goats. A new technique can distinguish species of even closely related fossil bones using slight differences in masses of collagen fragments.

results Rob has gotten are something I've been dreaming of for 35 years." Touwaide predicts that such work will ultimately offer a "new direction" for drug discovery. "We're doing applied history," he says.

Researchers in Copenhagen noted that Fleischer hasn't yet gotten long enough DNA sequences to identify every plant in the tablets to the species level, and some worry about contamination. But many were impressed. Ancient DNA researcher Ludovic

Game-Miners Grapple With Massive Data



Science, attack! Plotting data (above) from guilds in World of Warcraft—this one (left) is named Science—revealed the social evolution in that virtual world.

After describing his methods with dozens of mathematical formulae, Christian Thureau's next slide shows the result. It looks like a pile of fine metal dust in a magnetic field, revealing the invisible lines of force. This plot comes from the kind of data set that social scientists dream about: a flawless digital record of the social behavior of more than 10 million people interacting in a highly controlled setting over a 4-year period. But Thureau, a computer scientist at the Fraunhofer Institute for Intelligent Analysis and Information Systems in Bonn, Germany, never observed any of his research subjects, at least not in person. He harvested the data from World of Warcraft (WoW), the wildly popular online fantasy game.

It's called game-mining: digging for insights on human behavior in the terabyte-sized data logs generated by computer games. "You have over 10 million people playing World of Warcraft about 4 hours per day, 7 days a week," says Jaideep Srivastava, a computer scientist at the University of Minnesota, Twin Cities. "And that's on average; some play 80 hours per week!"

Because players' interactions are automatically recorded in WoW and many similar virtual worlds, researchers can use these massively multiplayer online games as natural laboratories. But the data are a challenge to interpret. Thureau's WoW study is a case in point. His goal was to reveal the evolution of WoW's guilds, the groups that players volun-

tarily form with each other to socialize, share resources, and slay monsters. Just the basic demographic information associated with the guilds amounted to 192 million 70-dimensional data points that represent information on the levels, skills, and activities of the players. "How do we make sense of that?" asks Thureau.

After failing with the classical techniques for finding patterns in high-dimensional data sets, he turned to a mathematical tool called archetype analysis, developed in the 1990s for physics and economics research. The original method failed at first because its computing time grows exponentially with the size of the data set, but Thureau devised a mathematical shortcut. The method works by identifying the most extreme data points—in this case, the guilds that are most different from each other—and describes the rest of the guilds as combinations of these archetypes. "It turns high-dimensional data into something that makes sense to humans," says Thureau.

CREDITS (LEFT TO RIGHT): WORLD OF WARCRAFT; CHRISTIAN THURAU

Killer Bots Are Getting Human

It was standing room only in the computer lab as intense violence played out on a giant screen. The game was Ultimate Tournament 2004, the classic multiplayer first-person shooter. But not all of the avatars blasting at each other were controlled by humans. Half of them were bots programmed by scientists in the room, nervously monitoring their programs for crashes. This was the third annual 2K BotPrize, a competition to create artificially intelligent game-playing agents that can fool a judge into believing they are human.

The contest is a variation on a classic test, first proposed in 1950 by computing pioneer Alan Turing, in which a judge has a conversation with a human and a computer and must decide which is which. The Turing test still defeats artificial intelligence (AI) 60 years later; machines largely remain terrible conversation partners.

Action-based video games can offer an alternative Turing test. "They

don't require speech, they provide a highly constrained environment but are still a challenge for AI," says Philip Hingston, the computer scientist at Edith Cowan University in Perth, Australia, who organized the contest. The rules are simple: Avatars controlled by humans and bots are dropped in a complex environment littered with weapons. It's kill or be killed. Each round, some of the human players—the judges—must decide which of their opponents are machine-controlled, based solely on their behavior. The team that designed the bot best at fooling the judges wins the \$5000 prize and a trip to Australia, funded by the game company 2K.

This year's prize was scooped by Conscious Robots, a team of Spanish computer scientists. Its bot represents a leap forward for game AI, says Hingston, because the team used machine consciousness, a technique rarely applied because of its complexity. Rather than just mimicking human behaviors—such as using imperfect aim or introducing randomness into running routes—the team's bot was designed to think like a human. "In our approach, we try to effectively integrate several cognitive

The output was an eight-dimensional “shadow” of the WoW data, projected as a simple 2D plot, that evolves over the course of the 4-year period of the study (see figure, p. 30). For the most part, says Thureau, the results confirm many researchers’ hunches about the social behavior of WoW players. Only a small fraction of guilds are active, those run by highly organized, ambitious groups of players. In spite of the staggering fraction of their lives spent in the game, most players are “casual rather than hardcore,” he says.

Game-mining isn’t just for multiplayer games. A team led by Georgios Yannakakis, a computer scientist at the IT University of Copenhagen, described player behavior in *Tomb Raider: Underworld*, a single-person game in which a gun-toting female archaeologist steals artifacts from ruins. They analyzed data from 10,000 players on the Xbox Live network, covering 35 different variables such as the use of weapons, the rate of progress, and whether it was tigers, traps, or other hazards that killed them. Their aim was to train a computer to predict the level at which any given player will eventually quit the game out of frustration—one of the hopes of the game industry is to create “personalized” games that adapt to each player’s abilities and interests.

The computer wasn’t perfect at foretelling the players’ fates, but it was far better than random. Just by observing how people played the first two levels of the game, it could predict with 77% accuracy where they would give up. Much of that prediction power came from counting the number of seconds players took to navigate a single obstacle, the jellyfish-filled “flush tunnel.” Yannakakis says the accuracy should improve as he tracks more players for the training, as well as obtaining “finer granularity” in the data, such as players’ exact routes of movement.

—J.B.

Smarts for Serious Games

You are a firefighter. As a blaze spreads across the factory, a paint canister goes off like a bomb. There are still panicked workers to be cleared. And to make matters worse, one of your crew is injured. How do you proceed?

Don’t worry, it’s just a game. But playing it could save lives. Games with ulterior motives such as teaching or training people—known among researchers as “serious games”—are on the rise, providing a cheap and safe supplement to on-the-job training. But serious games face “big problems” because of their simple programming, says Joost Westra, a computer scientist at Utrecht University in the Netherlands. In a real fire, there can be hundreds of people making unpredictable decisions all around you. Yet the nonplayer characters (NPCs) in the games usually follow tightly scripted behaviors, so unless you play exactly as the programmer expects, NPCs behave like confused robots. Another flaw in serious games is that they use “fixed scenarios or simple rules to determine the course of the game,” says Westra. “Expert users can quickly estimate how the game will react to their actions” but still must play through the easy levels to reach their proper level. The result, he says, is “disengagement, boredom, and possibly quitting the game before that level is reached.”

To fix these problems, Westra created a new architecture for serious games that uses artificial-intelligence (AI) techniques similar to those in some of the latest video games. He focused on a game called *RescueSim*, a serious game for firefighters. Rather than following scripts, Westra’s code turns each NPC into an autonomous agent with its own nuanced goals, responding to events as they happen. An NPC firefighter, for example, will have the

goal of extinguishing a fire but can switch to helping an injured comrade if no one else is near. An NPC’s awareness of what the game’s player and the other agents are doing is crucial, Westra says, because firefighting requires teamwork. One firefighter must turn on the pump while another keeps doors closed to prevent drafts that feed the fire; yet another must operate the hose.

In early testing of the system, the AI architecture shows promise. Not only does it make NPCs act reasonably, Westra says, but the entire game can also now adapt to different users. Beginners take on only simple jobs while NPCs take care of the rest; expert play-



Trial by fire. A so-called serious game trains rescue workers at a factory blaze.

ers must learn to command a crew in complex situations. “A game needs to be built with this architecture from the beginning,” says Westra, who plans to design a “bush fire team training” game with collaborators in Turkey.

“This is the future of serious games,” says Kyong Jin Shim, a computer scientist at the University of Minnesota, Twin Cities, who is developing such a system for training U.S. soldiers. “We need smarter agents and in-game characters.”

—J.B.



The humans are dead! A Spanish team (right) won this year’s 2K Bot-Prize.

skills, like attention and learning,” says Raúl Arrabales Moreno, a computer scientist at the University Carlos III of Madrid. The bot has a set of innate behaviors that are regulated by a higher control system, similar to the role of a conscious mind. It was incorrectly identified as human by the judges 32% of the time. By comparison, one human player was incorrectly identified as a machine 35% of the time. “There is only a slender gap between the humans and bots now,” says Hingston.

“There has been significant progress since the 2009 competition,” says Simon Lucas, a computer scientist at the University of Essex in the United Kingdom and one of the human players in the contest. Besides creating more engaging computer-controlled opponents for mass-market video games, the goal is to create better AI agents for “serious games” that simulate natural disasters and other complex problems (see above). Lucas predicts that a bot will be fully indistinguishable from human players “within the next 2 years.”

—JOHN BOHANNON



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LETTERS | BOOKS | POLICY FORUM | EDUCATION FORUM | PERSPECTIVES

LETTERS

edited by Jennifer Sills

Perennial Questions of Hydrology and Climate

THE POLICY FORUM BY J. D. GLOVER *ET AL.* ("INCREASED FOOD AND ecosystem security via perennial grains," 25 June, p. 1638) highlights environmental advantages of perennial relative to annual bioenergy crop systems but omits potentially important consequences related to hydrology and climate. They categorize greater perennial leaf area index and rooting depth (relative to annual crops) as "utiliz[ing] more precipitation," but the work cited provides no evidence for increased rainfall recycling.

The direct climate impact of land-use change associated with bioenergy expansion (such as a shift from annual to perennial cropping systems) has received little attention. The impacts of changing fundamental biogeophysical surface properties associated with bioenergy crops may have significant implications for local and regional climate (1). Changes to local hydrology caused by large-scale perennial systems may be complex, and thus require careful evaluation. For



Deep root systems. Perennials such as switchgrass deserve further study.

example, the drawdown of soil water (2) and enhanced evapotranspiration from perennial relative to annual cropping systems (3) could lead to long-term depletion of the soil-water column, as well as changes in clouds and rainfall in downwind locales. Quantifying local and remote consequences for hydrology and climate resulting from a shift from annual to perennial bioenergy crops is therefore required if long-term sustainability of biomass production is to be attained.

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Response

WE AGREE WITH GEORGESCU AND LOBELL THAT the effects of perennial bioenergy crops on hydrology and climate must be considered. However, our Policy Forum focused on the advantages of developing perennial grain crops (1), not bioenergy crops that can displace staple food crops.

We did not propose, as Georgescu and Lobell claim, a mechanism by which perennial crops utilize a greater portion of natural precipitation than annual crops. We also did not propose that perennial crops more efficiently produce biomass per amount of precipitation-derived water that is transpired. Shifting from annual to perennial food crops would likely have important consequences for how water is managed in agricultural landscapes, just as shifting from perennial-dominated native vegetation to annual crops has had dramatic, but generally detrimental, impacts. For example, the conversion of native forests to annual wheat production in southwest Australia disrupted the native hydrologic cycle, resulting in the rise of subsurface salts to the surface (2). Scientists there believe that

perennial grain crops could correct hydrologic imbalances by using more subsurface water, thereby reducing salinization while producing high-value crops (3). In areas of the world where annual crops often use only 10 to 30% of precipitation for transpiration (4), a shift to perennial food crops could increase the infiltration of precipitation into the soil by improving soil surface conditions (5), increase moisture retention in the soil by improving soil physical characteristics, and increase soil moisture available to deep root systems (6).

Perennial grain crop adoption would likely be advantageous in terms of climate change as well. Greater soil carbon storage and reduced input requirements mean that perennials have the potential to mitigate global warming. For example, perennial cropping systems have been shown to have net negative greenhouse gas emissions, whereas annual cropping systems tend to result in net positive greenhouse gas emissions (7). With more of their reserves protected belowground and with access to more soil moisture, perennials may also be more resilient to temperature increases predicted by some climate change models (8).

Adding grains to the inventory of available perennial crops would give farmers more choices in what they can grow and where, while sustainably producing high-value food crops for an increasingly hungry planet (9).

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A Positive Review for *PLoS ONE*

IN HER NEWS FOCUS STORY "FREE JOURNALS grow amid ongoing debate" (20 August, p. 896), J. Kaiser cites unnamed critics who accuse PLoS journals, particularly *PLoS ONE*, of publishing nearly all submissions, regardless of scientific value, simply to make as much money as possible.

These claims constitute a serious attack against open access publishing in general and against *PLoS ONE* in particular. As a member of the editorial board of *PLoS ONE*, I must say that, at least in my field (immunology), it is simply not true that the journal accepts bad science. Most of the papers I handle as an academic editor are of high quality and could qualify for acceptance by more conventional high-impact journals. At times, negative results are published, as they could be very important for other scientists in the field. If I am an expert in the field and totally convinced by the data of a manuscript, then I act as the reviewer and accept it (with any relevant suggestions for improvement). If I have doubts, I send

it out for peer review. If the paper is too weak, I reject it. This is exactly what editors of conventional journals do. *PLoS ONE* is a serious journal that aims to render well-performed science accessible to everybody.

JACQUES ZIMMER

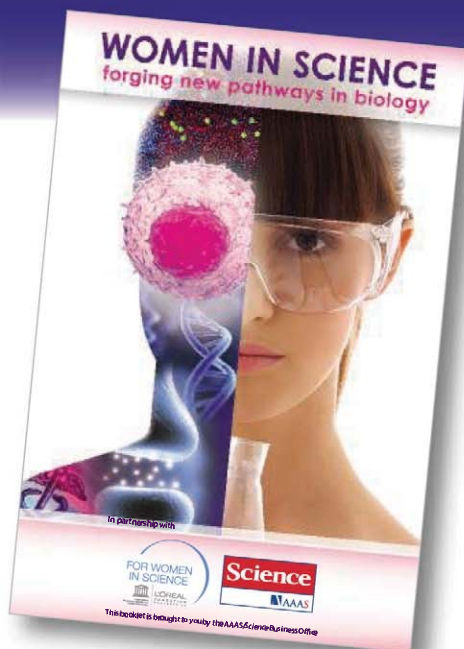
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HIV/AIDS: Use Existing Funds Effectively

THE ARTICLES IN THE SPECIAL SECTION ON HIV/AIDS: Eastern Europe (9 July, p. 159) omitted one important point: Considering the current economic downturn and limited resources available, it is particularly important to optimally use existing funds for HIV prevention and treatment. Economic studies such as cost-effectiveness analyses can inform decisions regarding the best resource allocation between available interventions. Research analyzing the public health and economic impacts of diseases and their interventions has become an essential tool for decision-makers; these results help them choose the

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most effective epidemic control programs while considering budget constraints.

To date, only a few scholarly articles have evaluated the cost-effectiveness of HIV interventions in Russia and Ukraine, in marked comparison to the scores of studies published on HIV in Africa. Scarce scientific informa-

tion may lead to inappropriate use of limited resources and further hamper efforts to contain the threat of a generalized HIV epidemic. Sound scientific evidence for the cost-effectiveness of debated interventions (such as opioid substitution therapy) could be a strong argument to accelerate adoption of

these programs. Studies on scaling up antiretroviral therapy, such as the case of injection drug users in Saint Petersburg, Russia (*1*), show that the interventions that reach injection drug users also prevent a substantial number of infections in the general population.

More attention from the research community should be directed toward studies of HIV in Eastern Europe. **SABINA STEFANIA ALISTAR**

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TECHNICAL COMMENT ABSTRACTS

Comment on "The Human K-Complex Represents an Isolated Cortical Down-State"

Florin Amzica

Cash *et al.* (Reports, 22 May 2009, p. 1084) argue that the human K-complex, a defining characteristic of slow-wave sleep, is a unipolar electroencephalogram (EEG) wave reflecting a simple neuronal hyperpolarizing event. We disagree with this conclusion and point to several confounding aspects of the study.

Full text at www.sciencemag.org/cgi/content/full/330/6000/35-a

Response to Comment on "The Human K-Complex Represents an Isolated Cortical Down-State"

Sydney S. Cash, Eric Halgren, Nima Dehghani, Andrea O. Rossetti, Thomas Thesen, ChunMao Wang, Orrin Devinsky, Ruben Kuzniecky, Werner Doyle, Joseph R. Madsen, Loránd Erőss, Péter Halász, George Karmos, Richárd Csercsa, Lucia Wittner, István Ulbert

Our study confirmed the hypothesis of Amzica and Steriade that the human K-complex (KC) shares neural mechanisms with so-called slow oscillation between periods of intense neuronal firing and silence but found that the KC can occur independently of this oscillatory activity. We agree with Amzica that the KC often has multiple components but contend that the major component is surface-negative and corresponds to the cortical down-state.

Full text at www.sciencemag.org/cgi/content/full/330/6000/35-b

Letters to the Editor

Letters (~300 words) discuss material published in *Science* in the previous 3 months or issues of general interest. They can be submitted through the Web (www.submit2science.org) or by regular mail (1200 New York Ave., NW, Washington, DC 20005, USA). Letters are not acknowledged upon receipt, nor are authors generally consulted before publication. Whether published in full or in part, letters are subject to editing for clarity and space.



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CYBERSPACE

Caught in the Net

Damian Tambini

Almost 600 years on, it seems clear that Gutenberg's invention of the printing press was a crucial factor in the rise of democracy in Europe and the decline of the old order of church and monarchy. Movable type and mechanized printing led to an explosion of free expression that was key to the emergence of modern pluralist democracy. Many claim that the historical impact of the Internet will be of a similar magnitude, that it will lead to an inevitable undermining of authoritarian regimes and the spread of democracy around the globe. In the words of early enthusiast John Gilmore, "The Net interprets censorship as damage and routes around it" (1).



But judging by the research and contributions gathered in *Access Controlled*, we will have to wait many more years before the nature of the impact of the Internet on dictatorship and democracy becomes clear. The authors provide an alarming range of evidence to support the view that authoritarian regimes are becoming ever more adept at controlling and censoring Internet communication. The volume raises a chilling possibility: that the early commentators were correct about the magnitude of the impact of the Internet on democracy—they just got the direction wrong. Could authoritarian regimes, and also democratic governments working with private companies, be perfecting a new form of authoritarianism, working with the grain of Internet communication and exploiting the intimate entwining of online communication with the everyday lives of citizens?

The research collected in the volume is the product of a path-breaking interuniver-

sity collaboration: the OpenNet Initiative. For the past five or so years, researchers around the world led by the Citizen Lab at the University of Toronto's Munk Centre for International Studies and Harvard's Berkman Center for Internet and Society have been attempting to reverse engineer a record of the search terms and content forms that have been censored or filtered out of Internet communication. The book, a follow-up to the editors' earlier *Access Denied* (2), explores in detail their findings, which are also available on www.opennet.net.

The contributors make abundantly clear that power has now followed people onto the Internet and has established a permanent garrison. First-generation controls, such as "Chinese-style national filtering schemes," were discussed at length in the OpenNet Initiative's previous reports and are still widely deployed in the more authoritarian countries. They involve developing databases of search terms and content keywords that are deemed threatening by the regime. These are removed from search results and from served information at key choke points in the Internet architecture. By conducting searches from within these jurisdictions, OpenNet researchers have found evidence of these practices in dozens of countries, most notoriously China and Saudi Arabia. As Ronald Deibert and Rafal Rohozinski note in their introductory chapter, second- and third-generation controls are more subtle and flexible. Through "outsourcing or privatizing," governments may have third parties "restrict what type of information can be posted, hosted, accessed, or communicated online." The authors discuss a variety of control techniques, including "the infiltration and exploitation of computer systems by targeted viruses and the employment of distributed denial-of-service (DDoS) attacks, surveillance ..., legal takedown notices, stifling terms-of-usage policies, and national information-shaping strategies." Such measures "reflect a maturation of methods resulting from a growing colonization of cyberspace by states and other actors."

In Russia, where the leading Web portal Yandex claims a readership greater than all the combined popular daily newspapers, much Internet use is focused on the RuNet: "a self-

Access Controlled

The Shaping of Power, Rights, and Rule in Cyberspace

Ronald Deibert, John Palfrey, Rafal Rohozinski, and Jonathan Zittrain, Eds.

MIT Press, Cambridge, MA, 2010. 633 pp. \$50, £37.95. ISBN 9780262014342. Paper, \$25, £18.95. ISBN 9780262514354. Information Revolution and Global Politics.

contained linguistic and cultural environment with well developed and highly popular search engines, Web portals, social network sites, and free e-mail services." Deibert and Rohozinski describe Russian Internet control as a third-generation approach that combines soft censorship and state-sanctioned moderation.

Access Controlled contains a sobering array of detailed and methodically gathered information, particularly on the filtering and blocking practices of formerly authoritarian countries. If there is a problem with the volume, it is that the subtlety of the third-generation controls makes them much harder to define. Many of the criticisms directed at third-generation techniques on RuNet could also be leveled at licensed broadcasters in established democracies. And as the list of practices makes clear, third-generation methods include controls imposed by private actors (is this still censorship?) and denial-of-service attacks that are often the work of individual hackers rather than states. It is true that states are becoming more subtle and are using these private enforcement agencies. Nonetheless, it is important to be precise about what constitutes censorship and to clearly distinguish between, for example, blocking or direct prior censorship of political content and notice and takedown of child abuse sites. Simply lumping both into the category of bad censorship doesn't convince.

Finding a normative terrain upon which to assess the impact of the Internet may simply be too huge a task—as the Internet becomes embedded in every aspect of social life, old terms (even "privacy" and "censorship") come under strain. To set up the debate in terms of the value opposition between freedom (generally good) and censorship (always bad) is politically useful but perhaps not analytically helpful.

So in addition to the excellent mapping work in *Access Controlled*, there is a more theoretical task to be completed: that of reassessing the value and meaning of the conceptual lexicon for dealing with issues of expression, censorship, and rights. In the era of mass media, an act of prior government censorship (through, for example, intimidating publishers or journalists, breaking presses, or refusing a broadcasting license) was relatively easy to detect and identify. Subtle legal distinctions have been honed that limit state censorship and identify justified and necessary restrictions. But in the converged Internet world, censorship is becoming much more difficult

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to identify and condemn. This explains some of the conceptual strains in this volume. As the Net continues to entangle power, rights, and rule in coming years, it is crucial that research monitors not only the technical and legal controls over Internet communication but also the normative and theoretical frameworks we use to evaluate them.

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10.1126/science.1196184

MUSEUMS: MAMMALS

Cenozoic L. A. Stories

Debra Pires

Who doesn't remember the first time they entered a natural history museum and dropped their jaw at a large, articulated skeleton of some megamammal—most likely a mastodon, mammoth, or African elephant—that greeted them in the rotunda? I have fond memories of museum visits from my youth that transported me to locations around the world, revealing the fauna and flora characteristic of often exotic sites. Museums of science and natural history have come a long way since the days of static dioramas, and the new Age of Mammals hall at the Natural History Museum of Los Angeles County does not disappoint. It inspires the wonder I felt when I was younger while incorporating an accurate depiction of mammalian evolution that visitors of all ages can grasp.

Although mammals have existed for over 200 million years, the exhibit concentrates on the Cenozoic (the “Age of Mammals”) and mammalian diversification over the past 65 million years. The hall in the museum's newly restored historic 1913 wing may seem small, but the curators have really packed a punch into the space. When visitors first enter, they are greeted by a cheetah mounted as if sprinting across the African plains, flanked by an articulated skeleton in the same pose. Nearby

Age of Mammals

John Harris, Curator

Natural History Museum
of Los Angeles County,
Los Angeles, CA.
www.nhm.org/site/explore-exhibits/permanent-exhibits/age-of-mammals

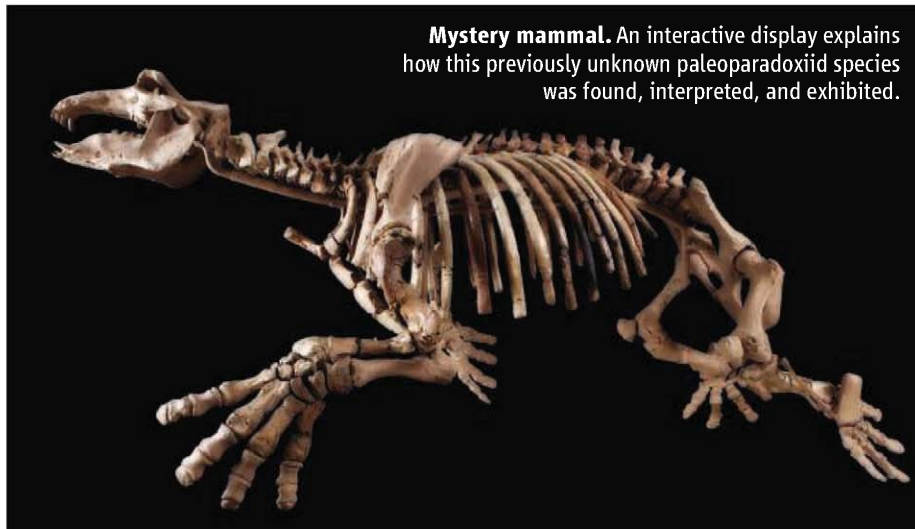
panels remind them of the characteristics that define mammals. These set the stage for presenting the evolution of mammalian diversity.

Beyond the cheetahs, the hall opens up to reveal a sample of exceptional taxidermy and skeletal specimens from the museum's collections. To help place the evolutionary diversification in context, an elevated display introduces three ideas at the heart of the exhibition: “Continents move. Climates change. Mammals evolve.” A video depicts shifting patterns of land masses, ocean currents, weather, and environments through the Cenozoic. The exhibition artfully ties the effects of past climate variation on mammalian evolution to our current concerns about climate change.

Interactive kiosks included in each major section of the exhibition allow visitors to examine changes in habitats over time, explore the relationships among different groups of mammals, learn more about the morphology and behavior of the animals, and quiz themselves about mammalian biology. The displays and interactive media seem quite effective at conveying information. Even those who have never had a course in evolution will probably find the phylogenetic tree of mammals easy to understand. As an educator, I was encouraged by watching children between the ages of 6 and 12 work on a topic at a touch screen until they had figured it out. The exhibit pays extensive attention to how mammals function, describing how differences in teeth reflect diet and how differences in limb posture reflect locomotion.

The exhibition's second part, arranged around the mezzanine, showcases the methods and tools that allow researchers to recon-

Mystery mammal. An interactive display explains how this previously unknown paleoparadoxiid species was found, interpreted, and exhibited.



struct the appearance and biology of mammals known only from fossils. Several of the displays here draw on material from the Page Museum at the La Brea Tar Pits. For example, one explores how paleontological data can be used to consider differences between animals that migrated through the Los Angeles region and those that were present year round. Demonstrating how tar pit remains can provide far more than a simple listing of the locale's Late Pleistocene fauna, this hidden gem of the exhibit effectively communicates the importance of collections and the process of science without directly stating either. A display of a 10- to 12-million-year-old paleoparadoxiid—an extinct, amphibious herbivore related to elephants—from Orange County offers an evocative description of the who, what, and how of paleontology. Here touch-screen activities allow visitors to uncover and identify fossil bones and prepare the specimen for display. Thus they will not only learn something about the regional paleofauna, they will better appreciate the nuts and bolts of what paleontologists do. Visitors will leave the exhibition's second part with a better understanding of functional morphology and how that can be used to infer characteristics of extinct mammals.

The Age of Mammals manages an ideal blend of the “wow” of Cenozoic mammals and the “how” of the science that underlies our understanding of them. With over 200 specimens, there may seem to be too much for people to absorb and enjoy. However, the presentation of the material reinforces the exhibition's central theme: shifting continents, changing climate, and altered habitats have shaped mammalian diversity both past and present. Visitors of all ages and backgrounds will find a museum experience that is both educational and enjoyable.

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Complex Systems View of Educational Policy Research

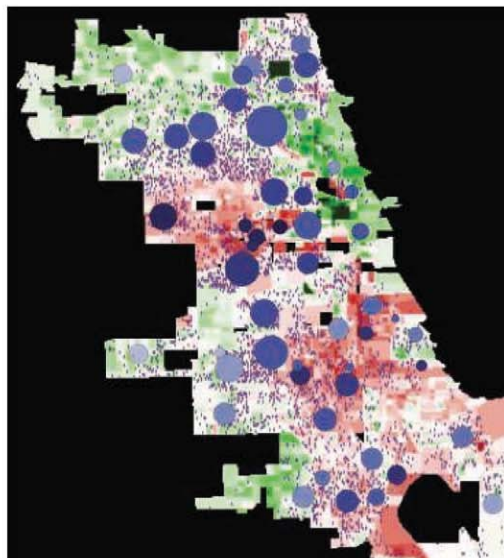
S. Maroulis,^{1,2,3} R. Guimerà,^{1,4} H. Petry,² M. J. Stringer,¹ L. M. Gomez,⁵ L. A. N. Amaral,^{1,6} U. Wilensky^{1,2*}

Education researchers have struggled for decades with questions such as “why are troubled schools so difficult to improve?” or “why is the achievement gap so hard to close?” We argue here that conceptualizing schools and districts as complex adaptive systems, composed of many networked parts that give rise to emergent patterns through their interactions (1), holds promise for understanding such important problems. Although there has been considerable research on the use of complex systems ideas and methods to help students learn science content (2), only recently have researchers begun to apply these tools to issues of educational policy.

We roughly categorize existing education research into two categories, “mechanism based” and “effects based.” Mechanism-based studies include ethnographies, case studies, and laboratory experiments that focus on understanding individuals and their interactions inside schools. Such work has provided insight into the motivation and cognition of students, teachers, and school leaders, as well as how social phenomena unfold inside schools (3, 4). Effects-based research treats factors contributing to academic performance of schools as inputs that work together to yield a particular level of student achievement (5). By analyzing variation in quantitative observational data on inputs and outcomes (6), or through the execution of field experiments (7), effects-based research has increased our understanding of the relative importance of factors such as teacher-pupil ratio, family background, and teacher quality and has established effects (or lack thereof) of specific interventions designed to improve achievement.

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ABM of school choice in Chicago. Students, represented by small dots, are shown in their census block. Dark red indicates high-poverty locations; dark green, low. Each circle represents a school. Color reflects the expected academic performance of students attending the school, given the experimental conditions of the model. Light blue indicates high mean achievement; dark blue, low. Circle size is proportional to expected enrollment. For more information, see the text and (32).

What Works, How It Works

Despite of such successes, and as evidenced by the call for more “mixed-methods” designs (8), a key challenge facing education research is to integrate insights about “micro-level” processes with evidence about aggregate, “macro-level” outcomes that emerge from those processes. For example, suppose we have results of a well-executed experiment using a nationally representative sample of schools that indicates students in small classes perform better than those in large ones. Although it is tremendously helpful to know that, on average, students in small classes do better, this alone is not enough to fully understand what changes policy-makers and school leaders should implement.

One reason for this is the issue of heterogeneous treatment effects (9). If small class size mattered under certain conditions but not others, school leaders would need to understand what happened differently in some small classrooms that led to better student outcomes. Both mechanism- and effects-based research may be helpful here, examining differing contexts and how programs are implemented. But we still face the challenge of aligning micro-level accounts with aggregate data. This is all the more difficult when considering inherent impediments to understanding complex systems: Effects are disproportional to cause; cause and effect are

separated in time and space; and properties of the macro-level system may be confused with properties of constituent, micro-level elements (e.g., attributing intelligence to individual ants when observing an entire ant colony intelligently gathering food) (10).

Additionally, we need to consider what are often referred to as “general equilibrium effects,” i.e., the systemic implications of class-size reductions enacted at a large scale (11). For example, partly on the basis of results of a randomized field experiment in Tennessee, California mandated statewide class-size reductions. However, many school districts had to hire teachers with limited training and credentials because the supply of qualified teachers was too small to handle the sudden increase in demand (12). If identified a priori, we can try to account for such effects using econometric models estimated from observational data (13). Although such models can often help characterize particular equilibrium states of educational systems at a larger scale, we are still interested in an additional, policy-relevant step: how to best move the system from one equilibrium state to another. Regardless of how well we account for heterogeneous treatment and general equilibrium effects, complex systems methods can help bridge these aggregate outcomes to underlying mechanisms at work in the system, as well as discover new and unanticipated systemic consequences.

Bridging the Gap

Applying a complex systems perspective to education research parallels the recent use of complex systems methods to model the spread of epidemics (14). Traditionally, one relied on (i) detailed case studies that traced social con-

tacts of a few infected individuals to identify the origin of an outbreak and elucidate infection mechanisms (15), or (ii) theoretical studies assuming large “perfectly mixed” populations where differences in infection mechanisms were simplified to aggregate measures of susceptibility and infectiousness (16). More recent work utilizing agent-based modeling (ABM), which allows modelers to “run” scenarios involving interconnected agents over discrete time steps to discover the emergence of macro-level properties, has helped link theoretical and case studies. Researchers now understand epidemics as macro-level outcomes that depend on relational, micro-level properties of the system, such as the structure of the contact network (17) and the reactions of agents to changing conditions (18). Such work has aided the development of antiviral drug distribution and quarantine strategies (19).

Although operationalizing rules governing individual behavior in educational systems may be more difficult than specifying micro-level rules of disease transmission, techniques for studying complex systems can complement more traditional approaches to education research in at least two ways. The first way is through visualization techniques, measures, and algorithms that facilitate network characterizations of social context. Although network characterizations are not new in social science (20–22), recent advances are particularly useful for education research. New tools for visualization of longitudinal network data enable researchers to connect fine-grained observations of classroom interactions, such as the content of student conversations, to emergent outcomes such as classroom discipline (23). Algorithms and measures developed to identify “communities” in networks (24) can identify boundaries of potentially influential social groups as they emerge from interactions driven by the local school context (as opposed to a priori categorizations of students into groups of “scholars,” “athletes,” etc.). Such techniques can be applied to widespread, existing data, enabling large cross-context analysis. For instance, one study used a network clustering algorithm to identify adolescent peer groups from class schedules and showed in a national sample of U.S. high schools that girls are more sensitive to social influence with respect to enrollment in mathematics courses (25).

The second way complex systems methods complement existing research is through the use of ABM, providing insights into how individual and group-level behaviors relate to systemwide phenomena (10, 26). To illustrate the potential of ABM, consider school choice

reform. Empirical research on programs that give households more choice is inconclusive, with methodological concerns arising for both observational and experimental studies (28). Computation has enabled work addressing the systemic effects, and related policy implications, of programs. For example, economists have used computational general equilibrium (CGE) simulations to identify features likely to minimize “cream-skimming” of top students by private schools in systems where government-issued vouchers are used to pay for private schooling (29).

ABM can extend such research by addressing questions pertaining to the paths between equilibrium points, such as whether a transition to choice might make a system worse before it gets better and for how long and for whom it is worse. Moreover, ABM enables investigation of a broader range of agent-level behaviors, including rules for students and schools that more directly correspond to behaviors observed from mechanism-based studies and agent-level data (30, 31). We have used student- and school-level data from Chicago Public Schools to initialize an ABM that allows households to choose among all public schools in a district (see the figure). In one set of computational experiments, we allowed for schools with a greater “value-added” (ability to increase student test scores) to enter the district and varied the manner in which students ranked schools. When students valued a school’s previous test scores much more than its geographic proximity, it became more difficult for new schools of higher, but initially unknown, value-added to survive. Consequently, a micro-level rule that one might surmise should aid district improvement (placing a relatively high value on school achievement) can also limit district-level performance in certain conditions (32). Future models could be used more directly to design school choice programs. For instance, by calibrating the model with more detailed information about the distribution of household decision-making rules, one could identify locations for new schools that would most increase district-level achievement.

By providing tools to characterize and quantify relationships between individuals and to investigate how individual actions aggregate into macro-level outcomes, a complex systems approach can help integrate insights from different types of research and better inform educational policy. Education research must establish not only what works but also how and why it works.

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10.1126/science.1195153

Neutrophils Find Smoke Attractive

Peter J. Barnes

Although neutrophils defend the lung against bacterial and viral infections, they also promote an inflammatory response that can lead to several lung diseases, including chronic obstructive pulmonary disease (COPD), severe asthma, cystic fibrosis, and acute respiratory distress syndrome. These diseases are difficult to treat with anti-inflammatory steroids (glucocorticoids) because they actually increase neutrophil survival. In addition, T helper 17 cells, which may drive neutrophilic inflammation in the lungs, respond poorly to glucocorticoids (1). So far, nonsteroid-based anti-inflammatory drugs that target neutrophilic inflammation have had limited use because of side effects (2). On page 90 of this issue, Snelgrove *et al.* (3) describe how a neutrophil enzyme, leukotriene A_4 hydroxylase (LTA_4H), controls inflammation, with implications for new therapeutic strategies to treat debilitating lung disorders.

Activated neutrophils may have several deleterious effects in the lungs. They release reactive oxygen species that are proinflam-

matory and also impair the anti-inflammatory actions of glucocorticoids. Neutrophils also secrete enzymes whose activities in the lung may cause emphysema: serine proteinases, including neutrophil elastase and proteinase-3, which degrade elastin fibers and stimulate mucus secretion, and matrix metalloproteinase-8 (MMP-8) and MMP-9, which break down elastin and collagen. Neutrophils themselves release factors that attract neutrophils, thereby perpetuating the inflammation that leads to COPD (2).

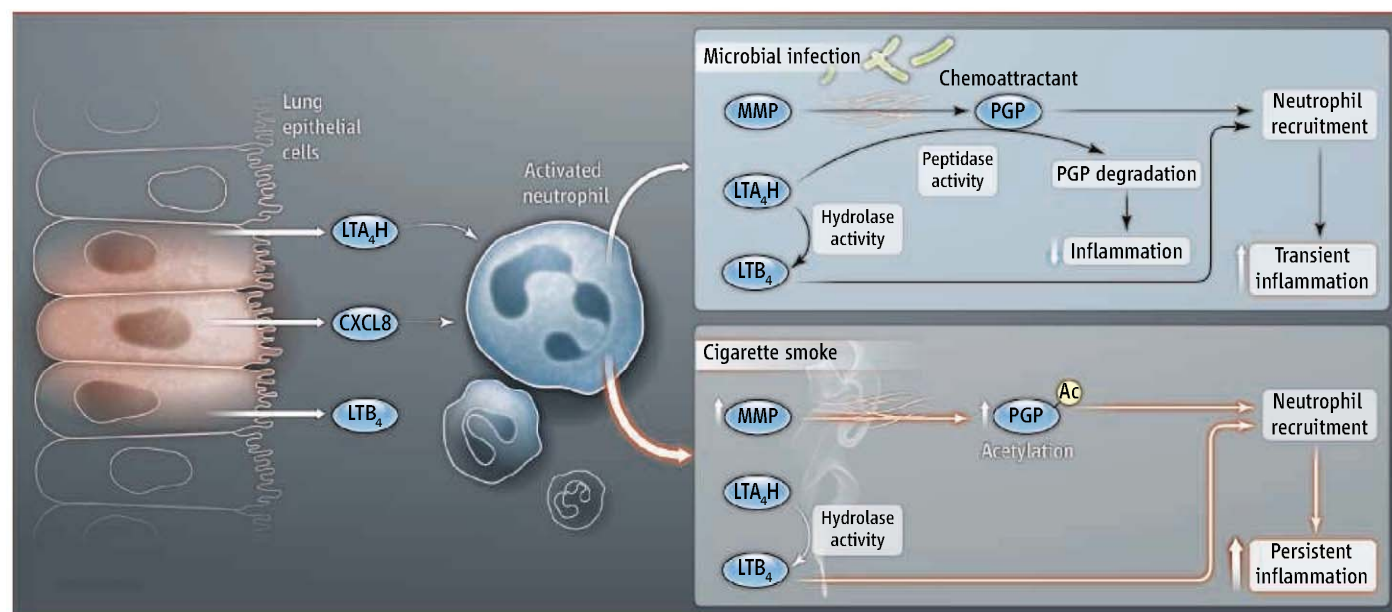
Neutrophils migrate into the lungs under the direction of chemotactic factors whose production is increased in the lungs of patients with COPD (2). These include leukotriene B_4 (LTB_4) and the CXC chemokines CXCL1 (GRO- α), CXCL5 (ENA-78), and CXCL8 (interleukin-8). CXCL8 signals through the neutrophil receptors CXCR1 and CXCR2, whereas CXCL1 and CXCL5 signal through CXCR2 (4). The relative roles of these factors have not been well defined, but an antagonist of the LTB_4 receptor and an antibody to CXCL8 reduce the chemotactic response of neutrophils in the sputum (respiratory tract mucus) of COPD patients by ~40% and 30%, respectively,

The altered activity of a neutrophil enzyme promotes the type of persistent lung inflammation seen in chronic pulmonary disease and cystic fibrosis.

and together by ~60%, suggesting that other chemotactic factors are also involved (5). A small-molecule antagonist of CXCR2 blocked the increase in sputum neutrophils after ozone exposure in normal individuals, pointing to CXCR2-activating chemokines as the predominant neutrophil attractants in the lung (6).

A selective neutrophil chemoattractant is the tripeptide proline-glycine-proline (PGP). PGP and its more potent acetylated form (N - α -PGP) are generated enzymatically from extracellular matrix proteins such as collagen (7). Indeed, PGP can be generated from collagen in vitro by MMP-8 and MMP-9 (8). Direct instillation of PGP or N - α -PGP into the lungs of mice induces neutrophilic inflammation and emphysema (9). PGP has also been detected in bronchoalveolar lavage fluid of patients with emphysema and in sputum from COPD and cystic fibrosis patients (9–11).

PGP appears to stimulate neutrophil chemotaxis through the CXCR2 receptor because its chemotactic effect is lost in CXCR2-deficient mice and by treatment of mice with antibodies that block CXCR2 (9). However, PGP does not appear to bind



Lung inflammation. In response to microbial infection or cigarette smoke, lung epithelial cells release factors that activate neutrophils. The neutrophils then release factors that increase the inflammatory response in the lung. During infection, the peptidase activity of the enzyme LTA_4H degrades a neutrophil chemoattractant peptide (PGP), which helps to resolve inflammation. However,

this enzymatic activity is inhibited by smoke, an agent that also stabilizes PGP through acetylation (Ac) of the peptide. In this case, neutrophil migration into the lung increases, leading to persistent inflammation and eventual chronic pulmonary disease. A similar mechanism may underlie persistent inflammation in cystic fibrosis and severe asthma.

CREDIT: Y. HAMMOND/SCIENCE

directly to CXCR2 because it fails to block CXCL8 binding to the receptor and does not activate the intracellular signaling cascade that CXCL8 elicits (12). One possibility is that PGP causes neutrophil chemotaxis indirectly, by provoking the release of CXC chemokines by the lung epithelium.

Snelgrove *et al.* report that PGP is normally inactivated in the lungs by the enzyme LTA₄H, which is released from neutrophils and epithelial cells (see the figure). This is entirely appropriate in the context of acute microbial infection of the lungs because neutrophilic inflammation resolves once the pathogen disappears. The authors show that acute infection of mouse lung with either influenza virus or *Streptococcus pneumoniae* is associated with self-limiting neutrophilic inflammation and undetectable PGP in the lungs. By contrast, in mice lacking the gene encoding LTA₄H, substantial PGP was detected in bronchoalveolar lavage fluid. Thus, by secreting MMP-8 and MMP-9, neutrophils trigger the production of PGP to attract more neutrophils. These very neutrophils also release LTA₄H to terminate the action of PGP and resolve inflammation. In addition to peptidase activity, LTA₄H has hydrolase activity that generates the chemoattractant LTB₄. Thus, LTA₄H has two opposing actions that control the inflammatory response during acute infection of the lung.

Snelgrove *et al.* further show that cigarette smoke extract increases acetylation of PGP (possibly by components such as acrolein) and inhibits the peptidase activity of LTA₄H (without affecting its hydrolase activity), making PGP resistant to degradation by LTA₄H. Because degradation of the chemoattractant is blocked, neutrophil chemotaxis is increased and prolonged by cigarette smoke. This mechanism may underlie chronic neutrophilic inflammation in the lungs of smokers. PGP and *N*- α -PGP are increased in the sputum of COPD patients (10) and can be generated from collagen by sputum extracts from COPD patients, which contain MMP-8 and MMP-9 (13). This could account for the greater and persistent neutrophilic inflammation in airways of COPD patients compared to normal smokers. These tripeptides are also increased in the sputum of cystic fibrosis patients. In this disorder, the concentration of extracellular chloride ions is low (due to a defective chloride ion channel in lung epithelial cells), which may reduce the peptidase activity of LTA₄H.

What are the therapeutic implications for these new findings? Blocking the activity of PGP and *N*- α -PGP may be useful in treating neutrophilic inflammation. This could be achieved by its complementary peptide arginine-threonine-arginine (RTR), which blocks the chemotactic activity of PGP and

inhibits PGP-induced emphysema in mice (14). Because the effect of PGP is mediated by CXCR2, small-molecule antagonists of this receptor (which are now in clinical development for treating COPD, cystic fibrosis, and other neutrophilic diseases) should be effective (6). However, drugs that block the production of LTA₄H, such as inhibitors of 5'-lipoxygenase or phospholipase A₂, would be less effective because they could increase the concentrations of PGP and *N*- α -PGP in the lungs and therefore overcome any potential benefit of blocking LTB₄ generation.

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ARCHAEOLOGY

When Humans Arrived in the New Guinea Highlands

Chris Gosden

People now inhabit every continent on Earth, with the partial exception of Antarctica. How and when this occupation unfolded is the subject of considerable debate, in part because the process raises questions about the development of human intelligence, adaptability, and technology. On page 78 of this issue, Summerhayes *et al.* (1) add to the discussion with a thought-provoking report on what may be the earliest documented human occupation of Sahul, the land mass that once joined Australia and Papua New Guinea (PNG). Stone tools and plant remains indicate that, at least 43,000 years

ago, people were living in PNG's high Ivane Valley and exploiting plants for food, and also altering their environment.

By at least 50,000 years ago, modern humans had occupied lowland rainforest and savannah habitats across southeast Asia. Then they crossed the open ocean to Sahul, which existed when sea level was much lower than it is today. Previous research has identified early human settlement sites along PNG's coast and documented evidence of highland occupation in the Ivane Valley dating to as early as 41,000 years ago (2). In 2007 and 2008, Summerhayes *et al.* identified seven more sites of early occupation in the valley. Within four layers of sediment, they found stone tools of various types and evidence of hearths, plant remains, and smashed animal

In a high valley, humans were using plants and felling trees nearly 50,000 years ago.

bone that could not be assigned to a species. Calibrated carbon-14 dating indicated that the oldest site was occupied between 49,000 and 43,000 years ago.

Today, a favorite slogan of PNG's tourist board is "expect the unexpected," and much would have been unexpected for humans arriving in the Ivane Valley during the late Pleistocene. Although it lies just 8° south of the equator, palynological evidence suggests that the valley has been a cold, difficult, and unpromising place to live at any time over the past 50,000 years. It sits at an altitude of 2000 m, above the limits of today's agriculture and has long been home to unusual species of plants (and probably animals). The environment would have posed a wide range of challenges to the tiny migrating groups of human

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hunter-foragers, who had somewhat restricted technologies. But they certainly had survival skills: The capability to map, remember, and experiment would have been key to their ability to navigate through this new physical and social landscape and to identify potentially useful, and possibly lethal, plants (3).

Although the evidence of plant use presented by Summerhayes *et al.* is scant, it suggests that the settlers were exploiting pandanus, which produces useful leaves and starchy fruit. They also used yams, which were probably growing in their natural range at lower altitudes. Given the altitude and cold of the Ivane Valley, however, it is unlikely that people lived there permanently. Instead, it appears that they were

moving and bringing with them plants to eat, but not to grow. Still, these people did not just take the ecosystem as they found it; they altered it. Summerhayes *et al.* found heavy “waisted” stone tools, which were probably used to clear forests. The Bobongara site on PNG’s Huon Peninsula has also produced slightly younger evidence of waisted tools, suggesting that limited tree clearing may have been relatively common across mainland PNG at this time.

The world of Sahul’s early hunter-foragers was very different from anything found in the region today. One can imagine small and highly mobile populations moving up and down the interior mountain chains of what is now PNG, engaged in small-scale clearance

Early arrivals. New evidence, including stone tools apparently used for clearing trees (*inset*), shows humans were in the Ivane Valley from 43,000 to 49,000 years ago and later migrated to off-shore islands.

and some movement of plants. This set the stage for what came next: Within a few thousand years, archaeological evidence shows that people were crossing the sea to the off-shore islands of the Bismarck Archipelago, as well as migrating into Sahul’s wet and semi-arid areas, now in Australia. By 20,000 years ago, sites such as Matenkupkum in New Ireland, PNG, suggest that people were introducing wild animals—such as wallabies and rats—into ecosystems naturally poor in animal protein. Some 9000 years ago, what we label as farming appeared in PNG’s highlands (above 1000 m), occurring in cleared landscapes and exploiting introduced plants and animals. Today, PNG’s highlands, particularly the western highlands, have some of the country’s densest populations and most intensive agricultural areas, and are surrounded by higher and lower regions with less dense populations. The extraordinary evidence presented by Summerhayes *et al.* provides insight into how this colonization began and testifies to the capabilities of these early human settlers.

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High in the highlands. The Ivane Valley sits some 2000 m above sea level and has long been a challenging place to live.

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CREDITS: (TOP INSET) SUMMERHAYES; (BOTTOM) SHAW/SUMMERHAYES

CELL BIOLOGY

How to STIMulate Calcium Channels

Michael D. Cahalan

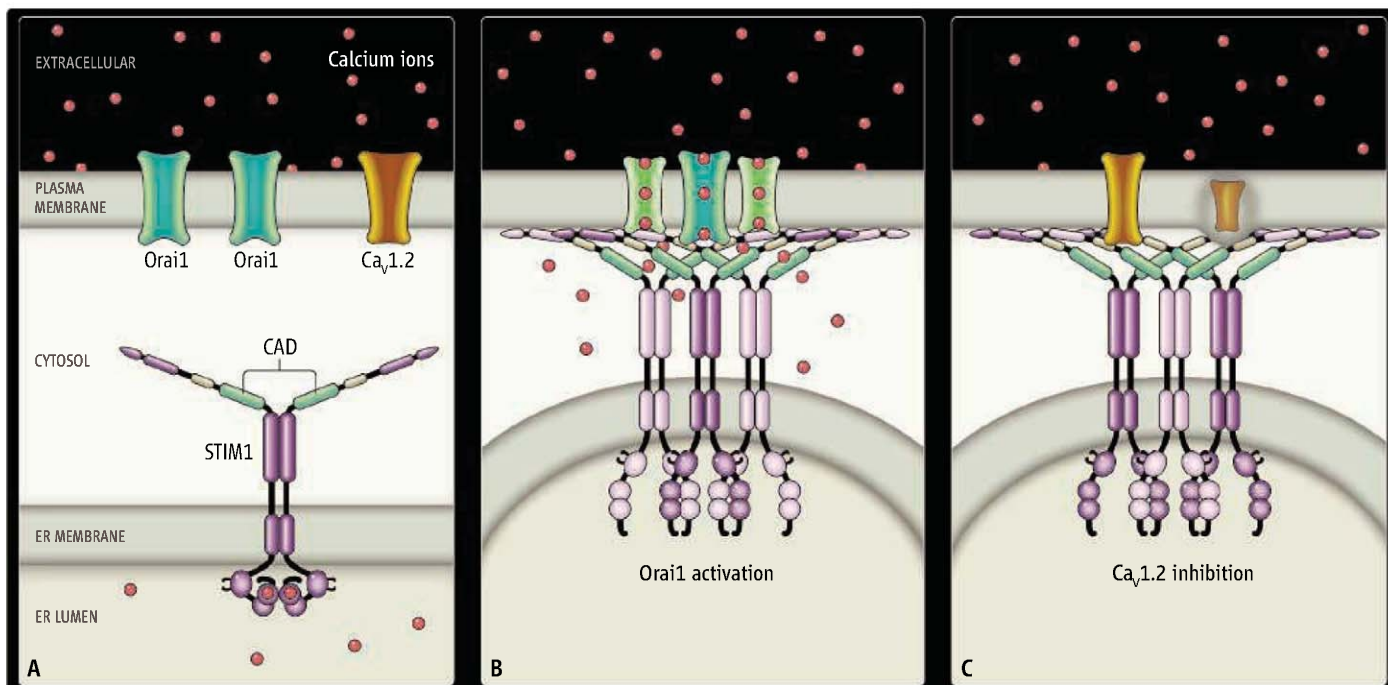
The movement of calcium across cell membranes regulates many important physiological processes in vertebrates, as various as the contraction of heart muscle, the firing of brain cells, the expression of genes by immune cells, and the secretion of hormones. Cells have two main types of calcium channels that enable calcium ions (Ca^{2+}) to flow into the cell. Voltage-gated Ca^{2+} channels typically function only in electrically excitable cells, such as neurons, heart muscle cells, and insulin-producing cells in the pancreas. By contrast, store-operated Ca^{2+} channels work only in electrically inexcitable cells, such as lymphocytes and other cells of the immune system. Researchers have long puzzled over this pattern, in part because biochem-

ical evidence suggested that both channel types are present in both excitable and inexcitable cells. On pages 101 and 105 of this issue, reports by Park *et al.* and Wang *et al.* (1, 2) help solve the puzzle by presenting a new mechanism that determines which type of Ca^{2+} channel predominates in a particular cell type. These two groups show that the protein STIM1, which was already known to activate store-operated Ca^{2+} channels, inhibits voltage-gated Ca^{2+} channels. Together, the reports help to illuminate a reciprocal calcium control mechanism that, if it goes wrong, can have life-threatening consequences.

STIM proteins first came to light in the Ca^{2+} signaling field 5 years ago; the name comes from their initial identification as a stromal-interacting molecule. Studies revealed that STIM proteins play an essential role in the function of store-operated Ca^{2+} channels in *Drosophila* (Stim) (3) and

Two studies reveal the reciprocal regulation of two different calcium channels.

in human cells (STIM1 and STIM2) (4). Later, researchers screening the *Drosophila* genome identified another family of proteins involved in the function of store-operated Ca^{2+} channels: the Orai proteins (Orai1, Orai2, and Orai3 in humans) (5–7). By subtly altering the structure of Orai proteins, researchers showed that they play a key role in calcium “selectivity,” or a channel’s ability to recognize Ca^{2+} ions and allow them to pass through a pore in the membrane (8–10). Together, these studies showed that STIM and Orai proteins work closely together to mediate the activity of store-operated Ca^{2+} channels. In general, STIM proteins function as a signaling relay; when Ca^{2+} stores are depleted at the endoplasmic reticulum (ER) within the cell, for instance, they can help to activate and open Orai channels in the outer plasma membrane (PM) (see the figure). Experiments have demonstrated



STIMulating. STIM1 in the ER membrane activates Orai1 (green) and inhibits $\text{Ca}_v1.2$ (orange) in the plasma membrane. (A) The cell at rest. Calcium ions (red) are abundant in the extracellular space and within the ER lumen but are rare in the cytosol. Functional domains of STIM1 include the EF hand (with calcium ions bound), and the CRAC-activating domain (CAD, green). (B and C) ER-PM junctions when the cell is activated and Ca^{2+} is depleted from the ER lumen. Calcium ions unbinding from

the EF hand of STIM1 trigger translocation of STIM1 to ER-PM junctions. The cytosolic junctional gap between ER and PM is sufficiently narrow (10 to 20 nm) to allow for direct molecular interaction of STIM1 with Orai1 and $\text{Ca}_v1.2$. The CAD of STIM1 recruits Orai1 to ER-PM junctions and opens Orai1 channels by direct binding to STIMulate Ca^{2+} influx (B). $\text{Ca}_v1.2$ channel proteins are also recruited to ER-PM junctions but are inhibited by the CAD interacting with the C terminus of $\text{Ca}_v1.2$ (C).

several functions of STIM proteins in activating store-operated Ca^{2+} channels (11).

In their studies, Wang *et al.* and Park *et al.* examined the role that STIM proteins might also play in regulating voltage-gated Ca^{2+} channels. Specifically, they examined one medically important member of this diverse family, known as the $\text{Ca}_v1.2$ subunit (α_{1C}). $\text{Ca}_v1.2$ forms the so-called L-type Ca^{2+} channels found in a wide array of cells, including those in the cortex, hippocampus, cerebellum, neuroendocrine system, heart, and arterial smooth muscle. Mutations in the $\text{Ca}_v1.2$ gene *CACNA1C* cause Timothy and Brugada syndromes, which are associated with life-threatening cardiac arrhythmias. A number of treatments, including the drugs verapamil, diltiazem, and various dihydropyridines, specifically target $\text{Ca}_v1.2$ channels.

The two groups found that the sequence of events involving STIM1 begins in the same way for both store-dependent and voltage-regulated channels, but the end result is very different. After Ca^{2+} store depletion in the ER, a structure known as the EF hand of STIM1 unbinds Ca^{2+} , and STIM1 oligomers translocate to form clusters immediately adjacent to the plasma membrane at ER-PM junctions. If voltage-gated Ca^{2+} channel proteins are expressed, they, like Orai subunit proteins, accumulate adjacent to STIM1. There, STIM1 and $\text{Ca}_v1.2$ interact via specific domains that protrude into the cytosol: a CRAC-activating domain (CAD) in STIM1 and the C terminus of $\text{Ca}_v1.2$. CAD opens Orai1 channels, but—as the two new papers show—it inhibits $\text{Ca}_v1.2$ channel activity in two ways: acutely (immediately) by physical interaction, and more slowly by causing internalization, a process that inhibits the channel and results in subsequent degradation of the channel protein. The two reports differ with respect to the relative importance of the acute and internalization phases (Wang *et al.* find that the acute phase is strong, Park *et al.* less so), but the outcome is the same: STIM1 regulates Orai1 and $\text{Ca}_v1.2$ reciprocally. In inexcitable lymphocytes, where STIM1 is relatively abundant, voltage-gated Ca^{2+} channels are inhibited and Orai1 is activated. In neurons and presumably other excitable cells, STIM1 is not so abundant and voltage-gated Ca^{2+} channel activity predominates.

As the activator for one Ca^{2+} channel (Orai1) and an inhibitor for another ($\text{Ca}_v1.2$), STIM1 assumes a new functional importance. In excitable cells, membrane depolarization (a change in electrical charge) activates Ca^{2+} influx through voltage-gated Ca^{2+} channels, but in lymphocytes depolarization actually inhibits Ca^{2+} influx through Orai

channels, because the electrical driving force for Ca^{2+} to enter is reduced. This potentially resolves a long-standing controversy regarding the type of Ca^{2+} channel in lymphocytes (12). Even if subunits of voltage-gated Ca^{2+} channels are expressed, they are not functional because of inhibition by STIM1. More important, STIM1's reciprocal functional interaction guarantees that only one type of Ca^{2+} channel or the other will be active.

Many questions remain concerning the generality of the findings. Does STIM1 block other voltage-gated Ca^{2+} channel family members? In native tissue, $\text{Ca}_v1.2$ subunits interact with numerous other cellular proteins that help with subcellular localization and modulation. Do they physically or functionally protect the channels from being inhibited by STIM1? When STIM1 is knocked out in mice or in rare patients with

mutations of STIM1, what are the consequences for voltage-dependent Ca^{2+} channel function in vivo? Answers to these questions should further improve our understanding of how Ca^{2+} signaling is regulated.

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CHEMISTRY

A Balancing Act for Taxol Precursor Pathways in *E. coli*

Tiangang Liu and Chaitan Khosla

A fermentation process that balances the activities of several bacterial and plant enzymes makes a precursor to an anticancer drug in substantial quantities.

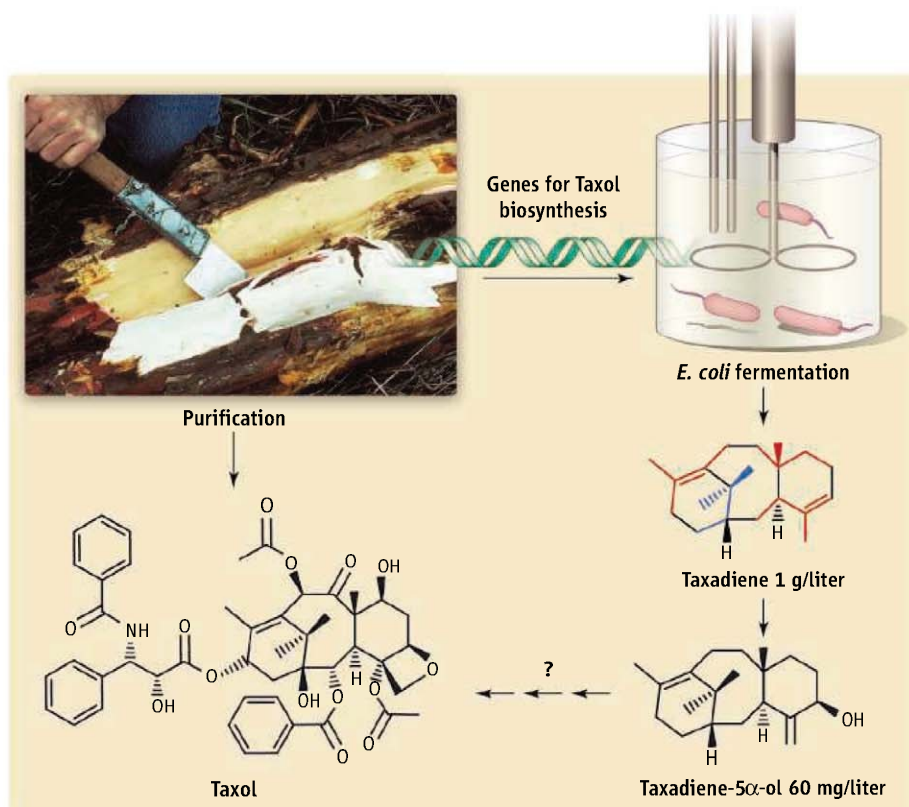
Taxol (paclitaxel) is a widely used cancer drug that was first isolated from the bark of the Pacific yew tree, *Taxus brevifolia*. In 1991, during early stages of its clinical use, 130 kg of Taxol were extracted from 1000 tons of bark, which required cutting down more than 500,000 mature Pacific yew trees (1). Fortunately, Taxol is presently made by less destructive methods, either through chemical conversion of a related molecule derived from needles of the more prevalent European yew, *T. baccata* (2), or from cultured plant cells (3). Nonetheless, both these plant-based processes are difficult, and this valuable drug remains expensive. On page 70 of this issue, Ajikumar *et al.* (4) have made progress toward making Taxol in *Escherichia coli*, biotechnology's workhorse bacterium, in substantial quantities by balancing several enzymatic pathways to make its complex multicyclic core (see the figure).

Taxol belongs to a large family of nat-

ural products called terpenes, which also includes artemisinin (a malaria drug), carvone (a flavoring agent), and pinene (the main constituent of turpentine). The carbon skeletons of all terpene molecules (taxadiene, in the case of Taxol) are made from two closely related five-carbon precursors, isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP), which are made in virtually all cells from substrates such as glucose. Each terpene skeleton then undergoes a series of further modifications by a set of enzymes that execute a sequence of reactions that leads to the complex natural product. The conversion of IPP and DMAPP into Taxol is a more complex process that so far has been seen only in cells from the Pacific yew bark.

Nature has two chemical processes for converting glucose into IPP and DMAPP. The "mevalonate pathway" operates in animal cells, as well as in microbes such as yeast. It was the target of a related recent effort aimed at reducing the cost of artemisinin manufacture (5). An unrelated process, the "nonmevalonate" pathway, synthesizes IPP and DMAPP in bacteria and plant cells.

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Rebuilding a biosynthetic pathway. The anticancer drug Taxol was originally obtained from the bark of the precious Pacific yew tree. Ajikumar *et al.* now report that a key intermediate in its chemical synthesis, taxadiene, can be abundantly obtained from *E. coli* by fermentation. The three terpene groups in the carbon-bond backbone from isopentenyl pyrophosphate are highlighted in red, and those from dimethylallyl pyrophosphate are highlighted in blue. The hydroxylation of this molecule to form taxadiene-5α-ol was also achieved, even though such reactions are usually difficult in fermentation processes. Further work will be needed to complete a chemical synthesis or an enzymatic pathway in *E. coli* that leads to Taxol.

Having chosen *E. coli* as their host for engineered Taxol biosynthesis, Ajikumar *et al.* targeted its native nonmevalonate pathway. They also genetically transplanted the first three enzymes in the Taxol pathway from the Pacific yew into *E. coli*. They developed a remarkably efficient fermentation process for taxadiene that also afforded respectable quantities of the next molecule in the Taxol pathway, taxadiene-5α-ol.

Specifically, *E. coli* synthesizes taxadiene with a volumetric productivity of 8 mg/liter per hour, and in lab fermentors, taxadiene accumulated to a final concentration of 1 g/liter. Previous attempts to make taxadiene in *E. coli* only yielded 1.3 mg/liter, with a correspondingly lower volumetric productivity (6). Although taxadiene-5α-ol only accumulated to a final concentration of 60 mg/liter, this is no ordinary accomplishment, in that chemical or genetic hydroxylation of inert hydrocarbons such as taxadiene is notoriously difficult. Several additional enzyme-catalyzed reactions are required to convert the taxadiene-5α-ol core into Taxol, although at least some of these transformations may be chemically feasible.

Multiple factors contributed to this engineering success. First, a dramatic improvement in the rate of IPP and DMAPP synthesis was attained through careful optimization of the absolute and relative levels of four key enzymes in *E. coli*. Second, the intracellular activities of two plant enzymes (GGPP synthase and taxadiene synthase) were enhanced substantially through codon optimization and deletion of an N-terminal plastid transit peptide. Third, the upstream segments to IPP/DMAPP and downstream segments to taxadiene were balanced by systematically varying promoter strength, plasmid copy number, and genotype. This endeavor appears to have set a new productivity standard in the field. Whereas most investigators typically choose optimal conditions by making and screening a relatively small number (<10) of alternative multi-genic configurations, the authors balanced carbon flux through the two segments with far greater precision by evaluating more than 30 such constructs.

For the final step, that of making taxadiene-5α-ol in *E. coli*, the authors needed to coax a cytochrome P450 oxy-

genase from the Pacific yew to work in a different cellular environment. In plants, these metalloenzymes partner with dedicated, membrane-bound cytochrome P450 reductases. Because such reductases cannot be expressed as readily in *E. coli*, Ajikumar *et al.* used a previously developed strategy (7) to engineer a chimeric enzyme harboring both oxygenase and reductase activities without a membrane anchor.

The authors also took advantage of an unexpected observation—the concentrations of an unrelated metabolite, indole, correlated inversely with taxadiene productivity. Follow-up experiments revealed that this inverse correlation primarily affected IPP and DMAPP productivity, and the authors exploited this empirical relationship as they optimized taxadiene biosynthesis. Their observation raises several intriguing and still unanswered questions. What is the mechanistic link between IPP/DMAPP and biosynthesis of tryptophan, the likely source of indole? Is this regulatory strategy unique to the present problem, or does it represent a more general mechanism for controlling carbon flow through a cell's metabolic networks? Finally, what are its implications for producing other useful chemicals through fermentation and other biotechnology routes? *E. coli* still has more secrets to reveal when it comes to designing the perfect chemical factory.

The same approach of Ajikumar *et al.* could be applied to a number of important terpene natural products derived from plants. Synthetic chemists can convert pinene into Taxol (8, 9) after many reaction steps, so there is no need to restrict possible targets to products that have been isolated from natural sources. Complex “green” chemicals could be made more affordably if feedstocks like taxadiene and taxadiene-5α-ol become commonly available.

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GENETICS

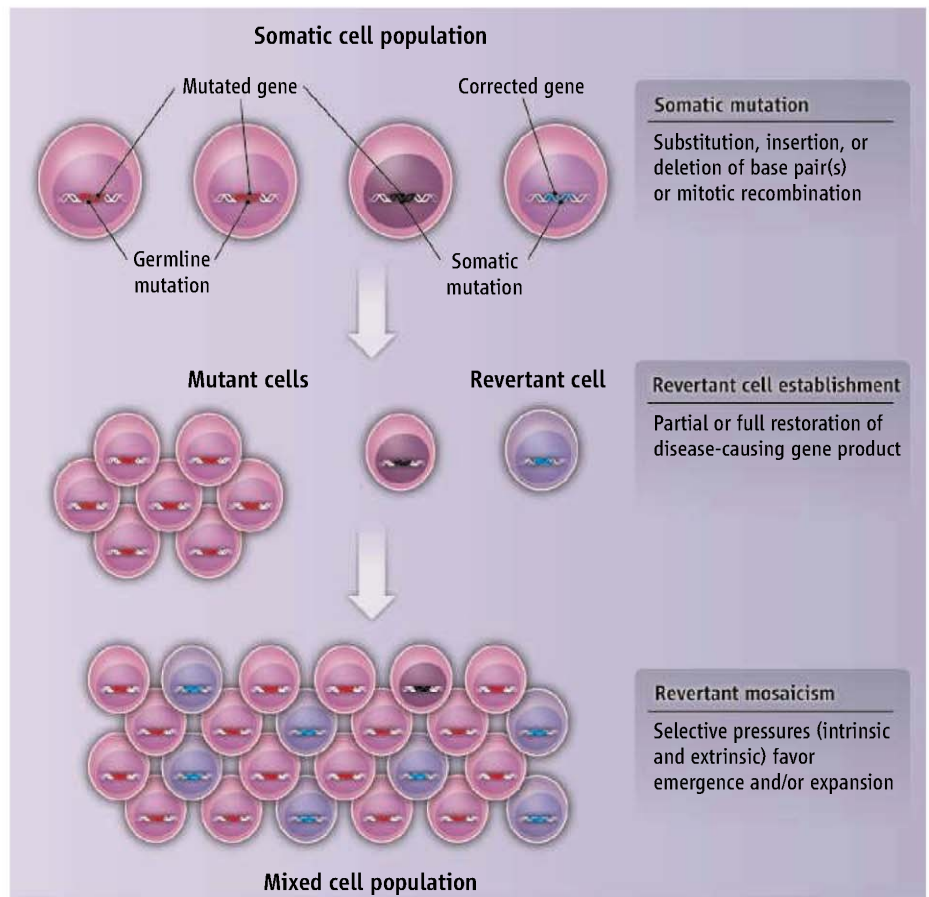
Mosaicism—Switch or Spectrum?

Brian R. Davis¹ and Fabio Candotti²

Somatic revertant mosaicism—the coexistence of cells carrying inherited genetic mutations with cells that have undergone spontaneous changes that correct the mutant phenotype—was previously thought to be extremely rare in terms of the frequency of patients in which it occurs and the frequency with which cells bearing revertant mutations arise in a given patient. A number of recent findings, including the report by Choate *et al.* on page 97 of this issue (1), challenge this perspective.

Somatic revertant mosaicism has been described in several genetic disorders affecting self-regenerating organ systems such as the skin, blood, and liver (2). Its development involves at least three steps: the occurrence of a correcting mutation in the already mutated gene; the survival of cells that have acquired partial or full functional restoration of the gene (revertant cells); and the selection and enrichment of these cells (see the figure). At present, little is known about the specific molecular mechanisms leading to revertant mutations (either during normal DNA replication or in response to genotoxic agents) or the precise selection processes for revertant cells. This limited knowledge derives from isolated reports of revertant patients carrying only one or a small number of revertant genotypes (2). As such, the commonly accepted picture has been that reversion is a highly inefficient stochastic process, possibly associated with unique characteristics of the patient exhibiting reversion.

Reports of multiple, independently arising, correcting, or second-site revertant mutations in the same patient have changed this notion and indicate that reversion is less unusual than had been thought. For example, revertant patches of healthy skin surrounded by the easily blistered skin in the disease junctional epidermolysis bullosa can arise from different molecular reversion events in the mutated genes (*COL17A1* or *LAMB3*) in the same patient (3, 4). This was not an infrequent occurrence; multiple revertant patches, each caused by a different molecular event, were identified in up to 30% of patients (5). Recent



Emergence of mosaicism. Somatic revertant mosaicism involves the three stages shown. Somatic mutations can arise in a disease-causing gene spontaneously or in response to DNA damage. If the mutation restores gene function (blue), this originates a revertant cell. If the revertant cell is capable of self-renewal and proliferation, selection pressures can act to favor expansion and detection. If the somatic mutation is not beneficial (black), the event is unlikely to rise above the detection threshold.

reports from primary immunodeficiency syndromes, such as the Wiskott-Aldrich syndrome (in which reversion occurs in 10 to 15% of patients) and severe combined immunodeficiency (caused by mutations in *CD3 zeta* or *RAG1* genes), have also documented multiple independent reversion events in patients (6–12). For example, at least 35 distinct revertant mutations were identified in one Wiskott-Aldrich syndrome patient (6, 7).

Taken together, these findings suggest that mosaicism develops through the ongoing generation of somatic mutations that affect the disease-causing gene in cells of self-regenerating organ systems. Although most of these somatic mutations will provide no benefit, some will result in partial or full functional restoration of the gene. Model cal-

A high rate of recombination restores the function of a mutated gene that encodes keratin, giving rise to the "confetti" spots in a rare skin disorder.

culations—taking into account the spontaneous DNA mutation rate and the number of cell divisions occurring in the newborn thymus—predict that cells that have corrections for specific immune gene mutations (e.g., correction of a stop codon) likely originate in most (or all) patients. A possible principal factor that distinguishes those individuals in which such events result in detectable revertant mosaicism is whether beneficial reversion mutations originated in a cell sufficiently early in its developmental pathway to provide for substantial expansion of revertant cells (such as epidermal stem cells for epidermolysis bullosa, and hematopoietic stem cells or lymphoid and thymic precursor cells for Wiskott-Aldrich syndrome), or perhaps otherwise in a particularly long-lived cell that is capable of expansion

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(such as a T lymphocyte for Wiskott-Aldrich syndrome). In this context, somatic revertant mosaicism is less likely a binary switch in which a given patient is either revertant or not. Rather, it appears to be a spectrum in which a patient originated revertant cells at some time, with the frequency, diversity, and functionality of the revertant cells dependent on various factors including the strength of selection. It is very possible that ultradeep sequencing methods that can detect rare gene variants can test the accuracy of this picture.

In their investigations on the skin disease ichthyosis with confetti (IWC, also known as congenital reticular ichthyosiform erythroderma), Choate *et al.* show that independent revertant clones, originating by loss of a heterozygous mutation on chromosome 17q, give rise to the thousands of normal skin spots that appear early in life in affected patients and increase in number and size over time. By mapping the location of common genetic recombination events in the revertant clones, the authors determined that dominant frameshift mutations in the *KRT10* gene cause the disease and discovered an unprecedented diversity of independent recombination events in humans. A common feature of

the various *KRT10* mutations is the expression of a mutant keratin protein that mislocalizes to the nucleus. Although another skin disease, epidermolytic ichthyosis, is caused by dominant negative or recessive mutations in the same *KRT10* gene, revertant clones have not been identified in this condition. As such, Choate *et al.* postulate a link between IWC mutant keratin 10 proteins and the high number of observed mitotic recombination events. If this is correct, increased mitotic recombination should affect chromosomes other than chromosome 17q. However, it may not be necessary to invoke a specific facilitating role of the mutant keratin 10 proteins in determining the occurrence of revertant skin patches in IWC. Indeed, the function of keratin 10 may be more severely affected by the IWC frameshift mutations than by the missense substitutions responsible for epidermolytic ichthyosis. If that is the case, revertant cells may be conferred a stronger selective advantage in IWC than in epidermolytic ichthyosis, thus allowing them to rise above the detection threshold by forming the “confetti” spots.

Somatic revertant mosaicism can be likened to a natural form of gene therapy, and the findings of Choate *et al.* have potential

relevance to therapeutic options for affected patients. These include using revertant stem cells (from patches of corrected skin) for engraftment; expanding preexisting corrected cells *in vivo*; and guiding efforts to induce targeted (site-specific) revertant mutations *in vivo*. For the latter, studies of revertant patients are particularly important, as they could inform the levels of correction that need to be achieved by gene therapy to obtain meaningful clinical effects.

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ASTRONOMY

A Dance of Extrasolar Planets

Gregory Laughlin

Launched in March 2009, the Kepler mission is tasked with searching for extrasolar planets. It continuously monitors 156,000 stars in a ~100-square-degree patch of sky covering a portion of the galactic disk centered on a direction lying in the constellation Cygnus (1). On page 51 of this issue, Holman *et al.* (2) report the discovery of a transiting planet whose orbit of 38.9 days varies by up to 1 hour due to the interaction with other planets in the system. This Saturn-sized world, known as Kepler-9c, circles a Sun-like star 2300 light-years away in the direction of the constellation Lyra, and is part of a bizarre system containing three transiting planets, whose mutual gravitational tugs generate an exquisitely choreographed orbital dance. Far from being mere curiosities, the planets of the Kepler-9 system may

provide vital clues to the mechanisms of planetary formation and orbital evolution.

Kepler can detect a 1/10,000 dip in brightness that occurs when an Earth-sized planet on an Earth-like orbit makes a ~12-hour passage (transit) in front of a Sun-sized star. The goal is to continuously monitor its target stars for a long enough period (at least 3.5 years) to observe repeated passages by Earth-sized planets on Earth-like orbits, leading to an estimate of the occurrence frequency of potentially habitable worlds.

Although Earth-sized planets have not yet been reported, Kepler has been remarkably productive in finding hot short-period planets with orbits ranging from days to weeks. In January of this year, its first five discoveries of four “hot Jupiters” and a “hot Neptune” were announced (3), and in June, an additional 312 candidate planets were reported (4). Although the members of this latest batch of planets require follow-up observations for individual confirmation, their bulk statistics

The Kepler space telescope has found a system of planets whose orbital timing varies due to gravitational coupling.

suggest that an important, and perhaps even the dominant mode of planet formation in our galaxy looks nothing like what happened in the Solar System: “Super-Earths” with masses between 5 and 15 times that of Earth are commonly found on orbits with periods of 50 days or less, with recent indications that up to a half of nearby Sun-like stars harbor objects of this type (5).

Kepler’s discoveries are contributing to a rapidly growing catalog of transiting extrasolar planets, yielding planetary masses, radii, bulk compositions, atmospheric constituents, and even weather reports (6). Furthermore, transiting planets in favorably constructed multiple-planet systems will exhibit measurable variations from strict orbital periodicity that can operate as detailed probes of the orbital dynamics (7, 8).

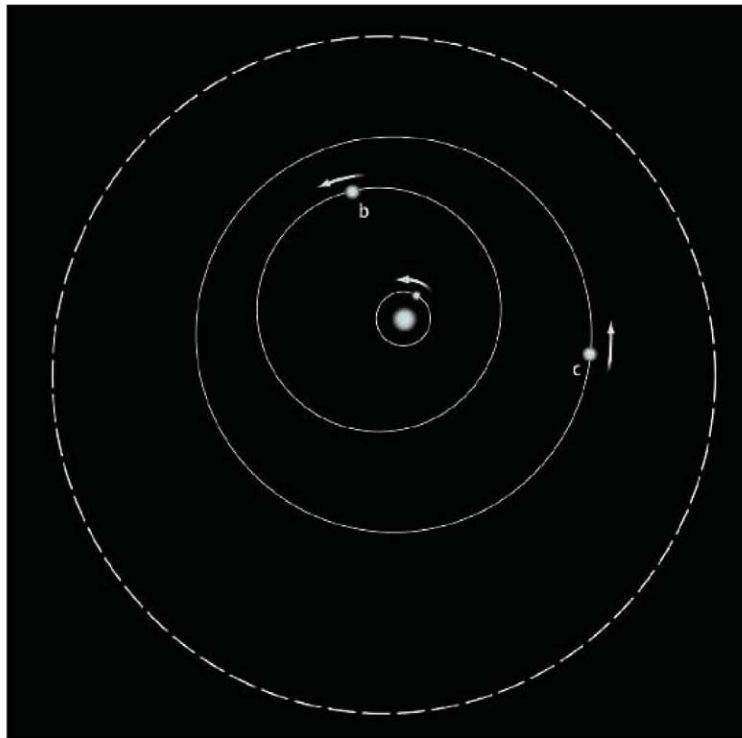
Although extrasolar transit-timing variations have proved elusive until now, astronomers have had centuries of experience with the transit-timing technique in our own Solar

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System. The Galilean satellites Io, Europa, Ganymede, and Callisto can all be observed in transit across the face of Jupiter, and in 1656, the Sicilian astronomer Giovanni Hodierna used transit-timing measurements to construct accurate predictive tables for the moons' motion. By mid-18th century, eclipse timing measurements for the jovian moons were sufficiently precise to demonstrate a curious relationship between the orbits of Io, Europa, and Ganymede. In 1743, the Swedish astronomer Pehr Wilhelm Wargentin published tables showing that the 1:2:4 ratio between the orbital periods of Ganymede, Europa, and Io is uncannily exact. Pierre Simon de Laplace, in 1784, demonstrated that the satellites' behavior could be understood purely in terms of their mutual gravitational interactions and that a specific combination of their mean orbital longitudes is governed by a tiny pendulum-like oscillation (or libration) about the perfect 1:2:4 commensurability (9). This behavior provides an example of what is now known as a mean-motion orbital resonance.

High-precision Doppler velocity monitoring has shown that resonant relations exist among a variety of extrasolar planets, notably the 1:2:4 resonance between the three outer planets orbiting the nearby red dwarf star Gliese 876 (10). The Kepler 9 system provides the first definitive detection of transit-timing variations and also represents the first detection of a system containing multiple separately transiting planets. The 19.2- and 38.9-day orbital periods of Kepler-9b and -9c imply that the two planets lie either close to or within a 2:1 mean-motion resonance (see the figure). Mutual gravitational interactions between the planets cause the inner planet's period to lengthen by an average of 4 min per orbit, whereas the period of the less massive outer planet is currently decreasing at an average rate of 39 min per orbit.

The transit-timing variations exhibited by Kepler-9b and -9c confirm unambiguously that the observed diminutions of the stellar light are caused by planets. Ordinarily, candidate planets detected by Kepler are arduously vetted to ensure that a transit-mimicking astro-



A planetary dance. The Kepler-9 system contains three transiting planets, whose orbits are shown here to scale, with the orbit of Mercury shown for comparison as a dotted line. Saturn-sized Planet "c" orbits the Sun-like primary star in 38.9 days and is in 2:1 resonance with similarly-sized Planet "b". Much closer to the star lies an Earth-size planet with an orbital period of only 1.6 days.

nomical phenomenon (such as the chance juxtaposition of a distant eclipsing binary star blended with a foreground star) is not responsible for the observed signal. The characteristic observed variations from strict periodicity, however, can only be caused by gravitational interaction, which provides both an immediate confirmation, as well as an accurate estimate, of the planetary masses.

The entire Kepler-9 system would fit within Mercury's orbit. But how did such systems come into being? Although it is possible that the planets were assembled in situ, it is more likely that they were formed further from the star, where both icy material and gas were readily available, and then migrated inward as a consequence of interactions with the parent disk. Kepler-9 has the potential to tell us a great deal about the system's history. The libration width and orbital eccentricity ratio between the outer resonant planets encode crucial information about the migration process. Over the next several years, a careful account of the transit-timing measurements will allow these quantities to be determined with great accuracy. Indeed, to date, only a single resonant exoplanetary system, Gliese 876, has been characterized to a degree where the libration width and the orbital eccentricity have been accurately

measured. Kepler-9b and -9c will provide a much-needed second example, and their transits will allow the physical properties of the planets themselves to be probed.

The detection and characterization of transiting extrasolar planets have engaged an extensive roster of astronomical assets, ranging from NASA's Kepler, Hubble, and Spitzer telescopes in space, to large ground-based facilities such as the Keck telescopes on Mauna Kea, and smaller, highly specialized facilities such as the European Southern Observatory's High Accuracy Radial velocity Planet Searcher (HARPS) spectrograph in Chile. Remarkably, amateur astronomers have been active participants in the ongoing quest, having codiscovered several of the most scientifically valuable transiting planets yet found (11, 12). The Kepler-9 system provides an excellent target for skilled backyard observers. During

the next several months, while the Kepler field remains visible in the Northern Hemisphere's autumn skies, observers will be able to obtain precise timing measurements of the upcoming transits (13) and get an advance look at the intricacies of this fascinating planetary system.

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10.1126/science.1196505

RETROSPECTIVE

George C. Williams (1926–2010)

Richard Dawkins

It has become a cliché that Charles Darwin would not have succeeded as a scientist today. He would not have won big research grants and did not have the mathematics to be a “theorist” by today’s conventions. But the cliché is wrong—for George Williams succeeded. Williams published books, rather than articles in “high impact journals”; he never won huge grants, did not head a big research group, and seldom used mathematics, yet he became one of the most respected figures in late-20th-century evolutionary biology. On 8 September, George Williams died at the age of 84.

Williams, like Darwin, was a world-class thinker. Like Darwin, he drew upon deep wells of knowledge; like Darwin, he wielded words and concepts with the precision that comes from clear thought; and like Darwin, he was correct far more often than a mathematical theorist might think he had any right to be. Also like Darwin, he was gentle and self-effacing, never aggressive or overassertive. He was a tall, rangy, patrician figure whose quiet, contemplative wisdom, as well as his dignified appearance, reminded many of Abraham Lincoln (Darwin’s exact contemporary to the day).

Williams received his Ph.D. in 1955 at the University of California, Los Angeles, and his academic path led him to Stony Brook University in 1960, where he remained for the rest of his career. He first attracted attention in 1957 with his evolutionary theory of senescence, a matured version of the idea sketched by Peter Medawar in 1952. Lethal genes that are expressed late in life outcompete those expressed early. Mutations that have good effects often have other effects too (pleiotropy), many of which will be bad. Natural selection will modify mutational effects such that the bad ones are postponed, and the good ones accelerated. Senescence follows, with the sort of clear logic that typified Williams’s thinking.

His 1966 book *Adaptation and Natural Selection* had a spring-cleaning effect upon evolutionary theory. Neo-Darwinism had fallen into lazy habits since the glory days of Ronald Fisher and the Modern Synthesis. The loose, intellectually shoddy idea of “group selection” was rife, and Williams

dispatched it. “Adaptations” were scattered throughout the literature with no understanding that adaptation is, in Williams’s memorably exacting phrase, “an onerous concept.” It can be studied with scientific rigor, but this first requires a clear answer to the question, “Precisely what is naturally selected?” Williams’s answer is compelling:

“The natural selection of phenotypes cannot in itself produce cumulative change, because phenotypes are extremely temporary manifestations... Socrates... may have been very successful in the evolutionary sense of leaving numerous offspring. His phenotype, nevertheless, was utterly destroyed by the hemlock and has never since been duplicated... The same argument also holds for genotypes. With Socrates’ death, not only did his phenotype disappear but also his genotype... because meiosis and recombination destroy genotypes as surely as death... It is only the meiotically dissociated fragments of the genotype that are transmitted in sexual reproduction, and these fragments are further fragmented by meiosis in the next generation. If there is an ultimate indivisible fragment it is, by definition, ‘the gene’ that is treated in the abstract discussions of population genetics.”

Quite so.

Williams efficiently disposed of “group selection,” which never recovered (except as a muddled version of kin selection). But in *Natural Selection: Domains, Levels and Changes* (1992), where he gathered many threads of thought, he developed the important and superficially similar idea (foreshadowed in *Adaptation and Natural Selection*) of “clade selection” to explain, not “altruism” but macroevolutionary patterns of diversity and—as I would put it—“the evolution of evolvability.”

One of Williams’s gifts was to show that what might seem obvious was not always so. He wrote *Sex and Evolution* (1975) “from a conviction that the prevalence of sexual reproduction in higher plants and animals is

The clear and wide-ranging vision of a great biologist enlightened our understanding of natural selection and adaptation.

inconsistent with current evolutionary theory... there is a kind of crisis at hand in evolutionary biology...” Assuming, as is common in animals, that the father contributes

less than the mother to the economic costs of rearing a child, a mutant female producing only asexual daughters would seem to be a more efficient gene-reproducing machine than her sexual rival, who pours up to half of her resources into economically unproductive sons. *Sex and Evolution* was the first book to wrestle with this paradoxical “twofold cost of sex”—constructively but not entirely successfully. After working through a number of models, including the “aphid-rotifer model,” the

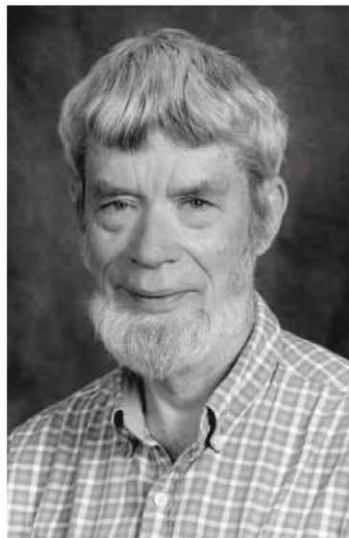
“strawberry-coral model,” and the “elm-oyster model,” Williams ended downbeat:

“I am sure that many readers have already concluded that I really do not understand the role of sex in either organic or biotic evolution. At least I can claim... the consolation of abundant company.”

Abundant and distinguished company, for the Williams “crisis at hand” was to provoke later books by, among others, John Maynard Smith and Graham Bell, and absorbed the great W. D. Hamilton (a somewhat similar character to Williams) for most of the latter part of his career.

Williams himself, in the latter part of his career, teamed up with the physician Randolph Nesse to found *The New Science of Darwinian Medicine* (1995). That is the subtitle of their excellent book, whose main title unforgivably mutated as it crossed the Atlantic (publishers do this lamentably often, and they did it with Williams’s only book for laymen). Darwinian medicine is too important for me to expound it briefly but, as I recommended on the cover, “Buy two copies and give one to your doctor.”

George Williams takes his place among the Darwinian immortals: like Darwin himself, a great scientist and a wholly admirable man.



High Frequency of Horizontal Gene Transfer in the Oceans

Lauren D. McDaniel,^{1*} Elizabeth Young,¹ Jennifer Delaney,¹ Fabian Ruhnau,² Kim B. Ritchie,³ John H. Paul¹

Microbes rely on mutation and the processes of horizontal gene transfer (HGT; conjugation, transformation, and transduction) to acquire new traits. Gene transfer agents (GTAs) discovered in the purple nonsulfur bacterium *Rhodobacter capsulatus* (formerly *Rhodospirillum rubrum*) are host-encoded viruslike elements that package random fragments of the host chromosome and are found in the genome of almost every sequenced member of the α -Proteobacteria order Rhodobacterales (1). To test whether GTAs are natural vectors of gene transfer, we grew nine strains of marine α -proteobacteria containing putative GTA cassettes (table S1) and screened them for the production of GTA-like particles.

Both *Roseovarius nubinhibens* ISM and the isolate *Reuveria mobilis* 45A6 reproducibly produced putative GTA particles during stationary phase growth. We then generated genetically marked donor strains of *R. nubinhibens* and *R. mobilis* containing the transposon Tn5. GTA production in these marked donor strains was equivalent to that of the wild-type strains. To document gene transfer frequencies, we subjected wild-type strains or natural communities from a range of environments to treatment with donor strain GTAs and documented the rates of GTA-mediated gene transfer of kanamycin resistance (fig. S1). In the coral reef environment, sponta-

neous kanamycin resistance was 4.6×10^{-4} , whereas the GTA-mediated frequency was significantly higher at 2.5×10^{-2} ($P = 0.028$, Student's t test).

For this experiment, both spontaneous mutants and GTA treatments were examined for the presence of the Tn5 streptomycin kinase gene. A total of 47% of the GTA-treated viable colonies but none of the spontaneous revertants contained the gene. That 53% of the putative transductants did not contain the gene is not surprising because these may have contained only the kanamycin resistance gene (*rptIII*) and not the flanking streptomycin kinase gene.

The recovery of the streptomycin kinase sequence, which is ~1000 base pairs (1 kbp) from the active site of the kanamycin resistance gene, suggested that up to 1 kbp of the central region of Tn5 was transferred. This is consistent with extracted DNA from the GTAs, which ranged from about 500 to 1000 bp in length (fig. S3). No spontaneous double antibiotic (kanamycin and streptomycin) resistance was detected, and the GTA-mediated frequency of 1.06×10^{-4} was significantly higher ($P = 0.023$). The Tn5 streptomycin kinase sequence was recovered in 1 in 10 viable double antibiotic-resistant strains, suggesting that modifications, truncations, or rearrangements may have occurred, as in natural transformation (2).

Similar frequencies of transfer were observed among differing environments (Table 1), demon-

strating that cultivated GTAs transduce natural communities of marine bacteria. The 16S ribosomal RNA sequences examined showed that the majority of natural GTA recipients were most similar to marine *Flavobacterium* or *Flexibacter* strains (table S2), consistent with the prior reports of abundant *Flavobacterium* in marine systems (3).

R. nubinhibens contains both a GTA and an inducible prophage (4). Transmission electron microscopy (TEM) demonstrated that *R. nubinhibens*-induced prophage preparations contained tailed phage (4), whereas GTA particles were nontailed (fig. S2A), resembling the GTA of *Silicibacter pomeroyi* (5). In contrast to the GTA particles, the purified prophages of *R. nubinhibens* had no gene transfer activity. Additionally, maximal expression of the *R. nubinhibens* GTA terminase gene cooccurred with maximal GTA production (fig. S4). TEM of GTAs of *R. mobilis* revealed tailed viral particles (fig. S2B).

GTA dose, or multiplicity of infection (MOI), was linearly correlated with increased resistance to antibiotics (MOI range from 0.01 to 10, $R^2 = 0.9593$), which enabled extrapolations of gene transfer frequencies to natural systems (6).

GTAs from *R. nubinhibens* ISM show a wide host range and interspecific gene transfer under ecologically relevant conditions. Environmental gene transfer frequencies ranging from 6.7×10^{-3} to 4.7×10^{-1} (Table 1) are 1900 to 459 million times the frequency for transformation (2) and 650,000 to 31 million times the frequency of transduction previously measured in the marine environment (7). These results suggest a genomic flexibility in marine microbial populations that facilitates their adaptation to changing environmental conditions.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/330/6000/50/DC1
Materials and Methods
Figs. S1 to S4
Tables S1 to S3
References

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Table 1. Frequencies of transfer of marker genes to both cultured and natural communities. N/A indicates not applicable; BDL, below detection limit.

Environment	Avg. spontaneous frequency	Range	Avg. GTA-mediated rate	Range	Number of trials
<i>Roseovarius nubinhibens</i> GTA filter matings					
Culture	6×10^{-7}	5.2×10^{-8} – 2.0×10^{-6}	1.7×10^{-5}	7.5×10^{-8} – 7.9×10^{-5}	$n = 5$
Estuary	1.6×10^{-4}	2.8×10^{-5} – 3.0×10^{-4}	8.9×10^{-4}	6.2×10^{-5} – 1.1×10^{-3}	$n = 3$
<i>Roseovarius nubinhibens</i> GTA liquid matings					
Estuary	1.2×10^{-3}	N/A	3.1×10^{-2}	1.2×10^{-2} – 5.0×10^{-2}	$n = 2$
Coastal	4.3×10^{-2}	N/A	2.8×10^{-1}	N/A	$n = 2$
Open ocean	2.5×10^{-2}	6.7×10^{-3} – 4.3×10^{-2}	3.9×10^{-1}	2.8×10^{-1} – 4.0×10^{-1}	$n = 3$
Reef	4.6×10^{-4}	N/A	2.5×10^{-2}	N/A	$n = 1$
Reef (double antibiotic)	BDL ($<10^{-6}$)	N/A	1.06×10^{-4}	N/A	$n = 1$
<i>Reuveria mobilis</i> (45A6) GTA liquid matings					
Estuary	1.2×10^{-3}	0 – 2.1×10^{-2}	2.4×10^{-2}	4.2×10^{-2} – 1.1×10^0	$n = 1$
Coastal	4.3×10^{-2}	3.0×10^{-2} – 5.6×10^{-2}	2.8×10^{-1}	1.6×10^{-1} – 6.4×10^{-1}	$n = 1$
Open ocean	3.3×10^{-3}	0 – 1×10^{-2}	4.7×10^{-1}	0 – 3.6×10^0	$n = 2$
Reef	4.6×10^{-4}	N/A	1.1×10^{-1}	N/A	$n = 1$

Kepler-9: A System of Multiple Planets Transiting a Sun-Like Star, Confirmed by Timing Variations

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The Kepler spacecraft is monitoring more than 150,000 stars for evidence of planets transiting those stars. We report the detection of two Saturn-size planets that transit the same Sun-like star, based on 7 months of Kepler observations. Their 19.2- and 38.9-day periods are presently increasing and decreasing at respective average rates of 4 and 39 minutes per orbit; in addition, the transit times of the inner body display an alternating variation of smaller amplitude. These signatures are characteristic of gravitational interaction of two planets near a 2:1 orbital resonance. Six radial-velocity observations show that these two planets are the most massive objects orbiting close to the star and substantially improve the estimates of their masses. After removing the signal of the two confirmed giant planets, we identified an additional transiting super-Earth-size planet candidate with a period of 1.6 days.

The Kepler mission was designed to measure the frequency of Earth-size planets in the habitable zones of Sun-like stars, by observing the dimming of star light when a planet passes in front of (that is, transits) its parent star (*1*). Transiting planets are particularly valuable, because their photometry, in conjunction with radial-velocity (RV) observations, yields the planets' physical properties (for instance, radius, mass, and density) (*2, 3*). Kepler has identified more than 700 transiting-planet candidates (*4*), including some systems with multiple transiting-

exoplanet candidates (*5*). Confirming that each of these candidates is actually a planet (as opposed to various astrophysical false positives; for example, diluted eclipsing binaries) requires extensive follow-up observations. We report the detection of a system of two transiting planets (Kepler-9b and 9c) showing transit timing variations (TTVs) (*6, 7*).

Kepler photometry. The Kepler data pipeline identified two transiting-planet candidates orbiting a star now designated Kepler-9 (KIC 3323887, 2MASS 19021775+3824032, KOI-377) and performed a series of checks to exclude common false positives (*8–10*). The Kepler photometric observations of Kepler-9 reported here span 13 May to 16 December 2009 universal time and consist of a nearly continuous series of 29.426-min exposures

through a broad optical bandpass (*1*). Because preliminary estimates of the transit times suggested that the transit times were not strictly periodic, this system was selected for intensive study.

The Kepler light curves (Figs. 1 and 2) show that Kepler-9 is a mildly active star, with photometric variations somewhat larger than those exhibited by the active Sun. The light curves show the effects of stellar spots (radius $\sim 0.1R_*$, where R_* is the stellar radius) and a stellar rotation period of ~ 16.7 days. Ground-based telescopic observations established that the properties of the host star are quite similar to those of the Sun [see the supporting online material (SOM)].

TTVs. The orbital periods of the two planet candidates are ~ 19.24 (Kepler-9b) and ~ 38.91 (Kepler-9c) days. We modeled each transit separately, allowing for variations in the transit time, duration, depth, and shape. Because there are substantial variations in the transit times but not in any of the other parameters, we determined the final transit times by fitting a detailed transit model, requiring a common transit duration, depth, and shape for each planet candidate, but fitting for the mid-time of each transit (Fig. 3 and tables S4 and S5). The observed changes are much larger than the ~ 80 -s uncertainty with which we measured the times of transit, as well as the expected variations due to a planet transiting stellar spots (SOM).

At BJD 2455088.212 (the weighted average of the transit times), the ratio of the best-fit orbital periods of Kepler-9c and Kepler-9b is $P_c/P_b = 2.023$, indicating that the system is probably affected by a 2:1 mean motion resonance (MMR). Resonance libration can cause the instantaneous period ratio to differ by a few percent from the long-term average ratio, even if the planets are well within the resonance. For planets in a 2:1 MMR, conservation of energy, assuming no strong scattering and slow variation of the orbital elements, implies that the period derivatives have opposite sign with a ratio dependent on the planet-planet mass ratio. We measure $-(\Delta P_b/P_b)/(\Delta P_c/P_c) = 0.375$, suggesting a mass ratio of Kepler-9c to Kepler-9b (M_c/M_b) ≈ 0.6 (see SOM). In addition to the slow change in orbital period, the times between successive pairs of transits of Kepler-9b also show an alternating pattern with an amplitude

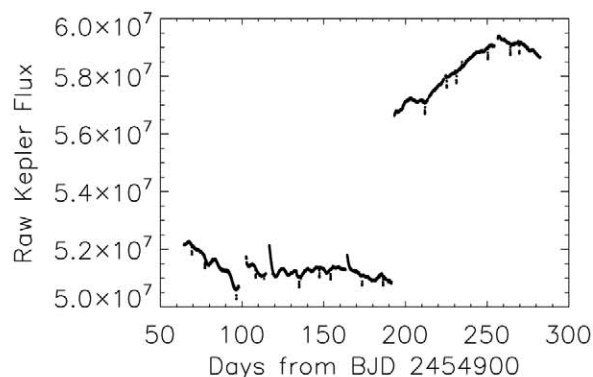


Fig. 1. The raw photometry of Kepler-9 (KOI-377, KIC 3323887) showing data from Quarters 1 to 3. The jumps in flux correspond to the breaks between quarters, when Kepler-9 moves to a different detector after the rotation of the spacecraft.

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of 2 to 4 min (Fig. 3). This “chopping” signal is due to the alternating position of the outer planet at the time of transit of the inner planet, and it scales with the mass of the outer body. These interdependent timing variations indicate that the two bodies are gravitationally interacting and, thus, must be orbiting the same star.

Dynamical model. The observed TTVs are the natural consequences of a system of two planets orbiting near the 2:1 MMR. We verified this by numerically simulating a fully interacting three-body system composed of a star of mass $1.0M_{\odot}$ (where $M_{\odot}$ is the mass of the Sun) and two planets. We fit the planet masses and orbits by comparing the transit times and durations predicted by the numerical model with those observed (Figs. 4 and 5).

Based on the photometry alone, we exclude masses for Kepler-9b that are less than $0.02M_{\text{Jup}}$ (where M_{Jup} is the mass of Jupiter) and greater than $4M_{\text{Jup}}$; for Kepler-9c, we exclude masses that are less than $0.03M_{\text{Jup}}$ and greater than $2M_{\text{Jup}}$, at the 3σ level (Fig. 4). This confirms that these are two planet-mass objects orbiting a Sun-like star and that their eccentricities are small (≤ 0.2). We verified that, for masses of Kepler-9b and 9c that correspond to brown dwarfs or stars, the fits to the transit times and durations are very poor, and the systems are dynamically unstable on time scales of tens of years.

Using the times and durations of transits observed over the lifetime of the Kepler mission, we expect that the TTVs alone will constrain the masses, eccentricities, and mutual inclination of the two established planets with substantially reduced uncertainties (SOM). Nevertheless, we obtained a few strategically timed high-precision RV measurements with the High-Resolution Echelle Spectrometer (HI) instrument on the 10-m Keck 1 telescope (SOM). Assuming that the RVs are dominated by Kepler-9b and Kepler-9c, we infer masses of $0.252 \pm 0.013 M_{\text{Jup}}$ and $0.172 \pm 0.013 M_{\text{Jup}}$ for Kepler-9b and 9c, respectively (Table 1), based on the combination of RVs, transit times, and durations (Fig. 4, dashed line). Figure 3 shows the RVs predicted by the model and those observed (these were included in the fit).

The measured ratio of transit durations is consistent with both planets transiting the same star with low-eccentricity orbits and similar impact parameters. Whereas the inclination of each planet’s orbit relative to the plane of the sky ($i_{b,\text{sky}}$, $i_{c,\text{sky}}$) is measured from the transit duration and shape, the relative inclination between the orbits depends on the difference in the longitudes of ascending node ($\Delta\Omega_{\text{sky}}$). In general, dynamical interactions induce nodal precession that, in turn, causes a drift in the impact parameter and the duration of the transits (12, 13). The lack of transit duration variations provides a constraint on the mutual inclinations and the presence of moons (12). Multiple transiting planets are most likely to be seen in systems with small relative orbital inclinations. For the orbital periods of Kepler-9b and 9c, the probability of both planets transiting

decreases rapidly as the relative inclination increases beyond 2° (14). The current observations favor a small relative inclination ($\leq 10^\circ$).

Follow-up observations. We performed a series of tests to exclude a variety of astrophysical configurations that could mimic the transits seen in the Kepler photometry. High-resolution imaging revealed that Kepler-9 has three neighboring stars located within 8 arc sec of itself, implying minor contamination ($\leq 1\%$) of the transit depth (15). Because all identified neighbors are ≥ 3.7 magnitudes fainter, none could mimic the Kepler-

9b or Kepler-9c transits, even if it were an eclipsing binary that dimmed by 50%. Moreover, the likelihood is negligible that the two transit signatures with a period ratio of 2:1 could be caused by stellar-mass companions, as this configuration would not be stable (16). Modeling of an exhaustive variety of stellar-blend scenarios (15), assuming the photometry of each planet candidate is the result of the brightness variations of an eclipsing binary being diluted by the brighter candidate star, has shown that, in the case of Kepler-9b and 9c, the only blends that could

Fig. 2. The detrended light curve of Kepler-9 (KOI-377, KIC 3323887), clearly showing two periodic transit signals well above the typical scatter of ~ 210 ppm. This was the light curve that we used to derive the results described in this paper. Kepler-9b, with a period near 19.2 days, is the slightly deeper transit, with the third and seventh transits missing (because of incomplete duty cycle). Kepler-9c, with a period near 39 days, is a little shallower, and all transits are observed. At this scale, the light curve of the third candidate, KOI-377.03, is not apparent.

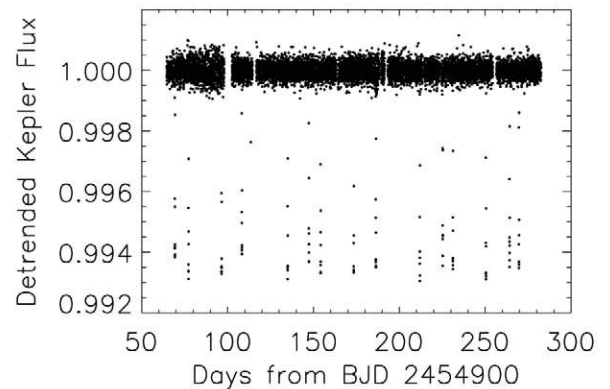
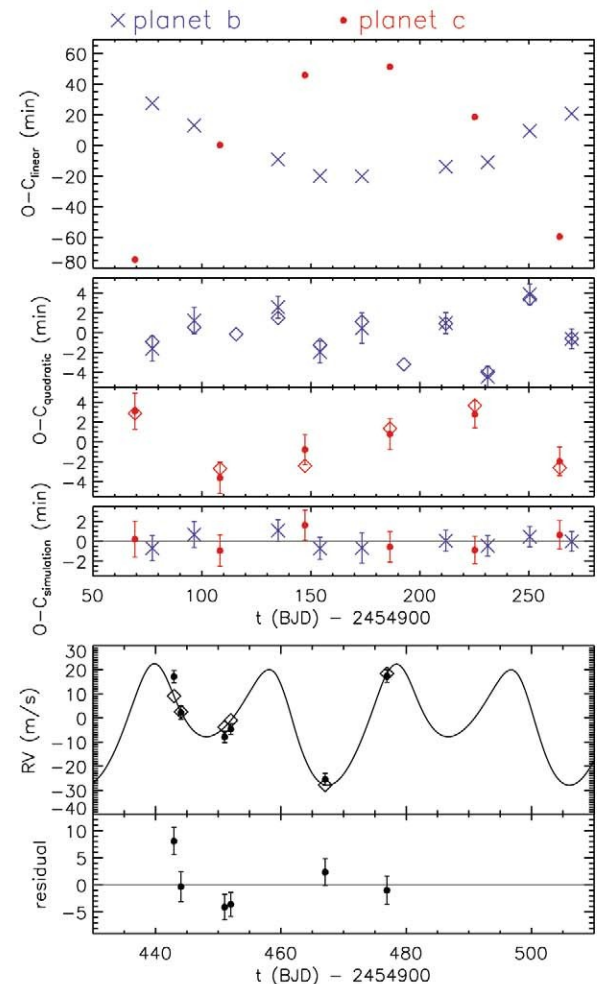


Fig. 3. (Top) Offset of the observed transit times for planets “b” (blue $\times$ symbols) and “c” (red dot symbols) compared to those calculated with linear ephemerides, quadratic ephemerides, and a dynamical model (diamonds) in which the planets fully interact. These calculations display the best-fit model (table S1). Only the diamond symbols are shown for events for which Kepler data were not available. **(Bottom)** A comparison of the observed RV (dots) with that predicted by the dynamical model (solid line and diamonds). Error bars indicate the uncertainties in the RV observations (SOM).



reproduce the Kepler light curve would be within two magnitudes of the target brightness. A combination of high-resolution imaging and spectroscopic constraints rules out these scenarios and provides an independent confirmation of the planetary status of the two planets.

Super-Earth-size candidate. After filtering out the transits of Kepler-9b and Kepler-9c, there is evidence for a third, smaller planet candidate with a period of 1.5925 days and a transit depth of ~ 200 parts per million (ppm) (Fig. 6). Until it is confirmed as a planet (as opposed to a background eclipsing binary), we will refer to this body as KOI-377.03, following the internal enumeration of Kepler Objects of Interest (KOI). Because the transit depth of KOI-377.03 is similar to the scatter in each 30-min observation, individual events are difficult to distinguish, but the transit light curve is very significant in binned photometry (Fig. 5). The transit duration (~ 2.8 hours) is consistent with a central transit of the same star orbited by Kepler-9b and Kepler-9c (which transit with a larger impact parameter). If this is a planet orbiting the same star, it would have a radius of $\sim 1.5 R_{\oplus}$ (where $R_{\oplus}$ is the radius of Earth), making KOI-377.03 one of the smallest planets detected to date. We verified with n -body integrations that a system composed of Kepler-9, Kepler-9b, Kepler-9c, and KOI-377.03 (assuming an Earth-like density for the KOI-377.03) is dynamically stable for at least the time scale of the numerical integrations (SOM).

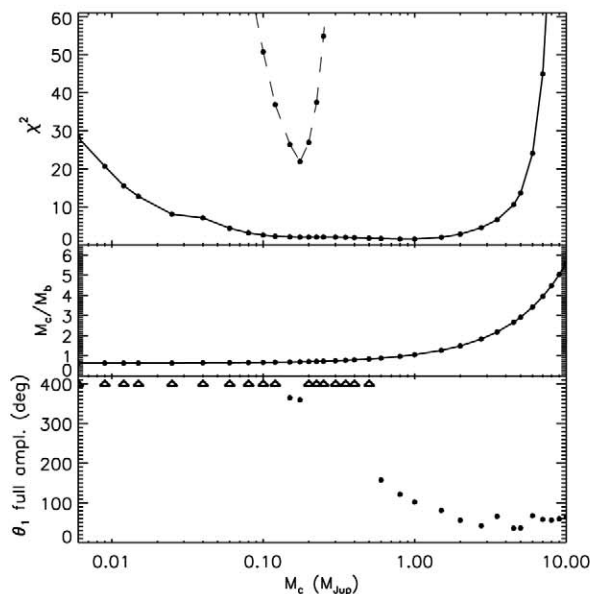
If KOI-377.03 is a planet in the same system, its expected TTV amplitude is only tens of seconds (7), which is not surprising given the large period ratio (12.1) between KOI-377.03 and Kepler-9b. Neither this signal nor the TTVs induced on Kepler-9b or Kepler-9c from KOI-377.03 are likely to be measured by Kepler. However, the expected RV signature from such a planet would be ~ 1.5 m/s semiamplitude (using an Earth-like

density) and may be contributing to the observed RV scatter.

Discussion. The detailed dynamics of a resonant system probes the system's formation. Resonant systems of giant planets inside the snow line most likely formed outside of resonance and at larger distances. Slow, smooth differential migration through the system's natal disk naturally leads to planets becoming trapped in the 2:1 MMR with small amplitude libration of resonant angles, as is the case for the GJ876 system (17, 18). Although several other planetary systems are near the 2:1 MMR (19–23) or show more complicated resonance structure (24), measuring the libration amplitude of resonant angles from RV observations is challenging, even for the most favorable system (24, 25). For multitransiting systems, on the other hand, resonant angles can be accurately measured via TTVs (26). For the Kepler-9 system, the expected libration period, based on numerical simulations, is ~ 4 years.

The best-fit Kepler-9b and 9c model is dynamically stable for more than 2.7 billion years, but only one of the resonant angles librates. For nearby, stable solutions, each of the resonant angles circulate. Large libration amplitude (Fig. 4, bottom panel) could be attributable to a more rapid migration (27) or excitation after resonant capture. For example, continued migration after resonant capture can lead to eccentricity excitation and, eventually, planet-planet scattering (28–31). Other mechanisms such as turbulence in the protoplanetary disk (32), scattering of planetesimals (33, 34), and perturbations by other planets (35) could also be responsible for a large libration amplitude. In principle, each of these mechanisms could perturb a resonant system so as to increase the libration amplitude. For Kepler-9b and 9c, tidal dissipation is expected to be too slow to have broken the MMR (36).

Fig. 4. Fits to the transit timing observations as a function of assumed mass of Kepler-9c (M_c). **(Top)** The χ^2 statistic, where the transit mid-times and durations are fit (solid curve), and the transit mid-times, transit durations, and RVs are also fit (dashed curve). **(Middle)** The ratio of planetary masses, showing that, for small masses, the mass ratio can be predicted from the ratio of the quadratic terms in the transit time ephemerides. M_b represents the mass of Kepler-9b. **(Bottom)** The full amplitude (ampl.) of the 2:1 resonance angle $\theta_1 = 2\lambda_c - \lambda_b - \omega_b$. λ_c is the mean longitude of Kepler-9c; λ_b is the mean longitude of Kepler-9b; and ω_b is the longitude of periastron of Kepler-9b in a 10^3 -year integration. Upward-pointing triangles indicate circulation of the resonance angle; dots indicate the full amplitude (in degrees) of libration.



Our models currently indicate a solar-mass star with a radius of $\sim 1.1 R_{\odot}$ (where $R_{\odot}$ is the radius of the Sun), based on the observed durations and impact parameters (SOM). Combining this observation with the well-measured radius ratio, the two planets Kepler-9b and 9c have nearly identical radii of $\sim 0.8 R_{\text{Jup}}$ (where R_{Jup} is the radius of Jupiter). The best-fit planet masses and radii are slightly smaller than those of Saturn. Theoretical models indicate that such planets are composed primarily of H and He (37–39).

The equilibrium temperatures for Kepler-9b and 9c are ~ 740 and ~ 540 K, respectively (assuming Bond albedos of 0.2). The mass-radius relation is insensitive to the equilibrium temperatures,

Table 1. Parameters for Kepler-9, Kepler-9b, and Kepler-9c based on the combined fit to the observed transit times, transit durations, and RVs. The quadratic ephemerides for both planets are approximations to the actual transit times and are not meant to be extrapolated beyond the observations presented in this paper. The quoted uncertainties in the planetary masses, radii, densities, and semimajor axes do not incorporate the uncertainty in the stellar mass (M_*). R_p , planet radius; b , impact parameter; M_p , mass of planet; AU, astronomical units; N , transit number.

Stellar parameters	Value
Mass M_* ($M_{\odot}$)	1.0 ± 0.1
Radius R_* ($R_{\odot}$)	1.10 ± 0.09
Planet "b" parameters	Value
Ephemeris (BJD)	$(2455073.43381 \pm 0.00052) + (19.243159 \pm 0.000098)N + (0.001274 \pm 0.000036)N^2$
Transit duration (days)	0.15927 ± 0.00044
Scaled planet radius R_p/R_*	0.07885 ± 0.00081
Scaled impact parameter b/R_*	0.654 ± 0.033
Mass M_p (M_{Jup})	0.252 ± 0.013
Radius R_p (R_{Jup})	0.842 ± 0.069
Density ρ_p (g/cm^3)	0.524 ± 0.132
Orbital semimajor axis a (AU)	0.140 ± 0.001
Planet "c" parameters	Value
Ephemeris (BJD)	$(2455164.18301 \pm 0.00074) + (38.908610 \pm 0.000738)N - (0.013452 \pm 0.000147)N^2$
Transit duration (days)	0.17121 ± 0.00057
Scaled planet radius R_p/R_*	0.07708 ± 0.00080
Scale impact parameter b/R_*	0.716 ± 0.026
Mass M_p (M_{Jup})	0.171 ± 0.013
Radius R_p (R_{Jup})	0.823 ± 0.067
Density ρ_p (g/cm^3)	0.383 ± 0.098
Orbital semimajor axis a (AU)	0.225 ± 0.001

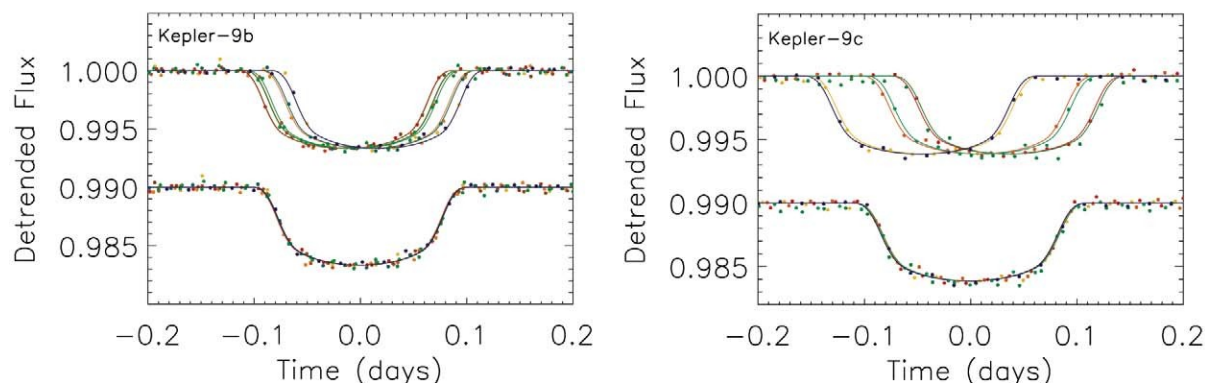
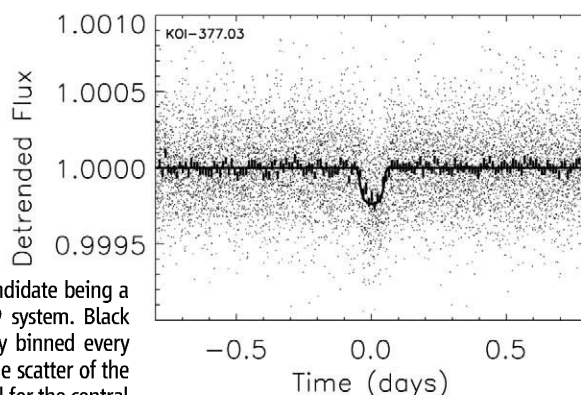


Fig. 5. Light curves for Kepler-9b (left) and Kepler-9c (right). In each panel, the top curve shows the detrended Kepler photometry (points, colored by transit epoch) folded with the best-fit period. Significant displacements are due to the large TTVs caused by gravitational interactions between the planets, as described in the text. The bottom curve in each panel (displaced

downward for clarity) shows the transits shifted to a common center using the measured transit times (SOM), giving depths of ~ 7 millimagnitudes and durations of ~ 4.5 hours. Also shown are solid lines (also colored by transit epoch) from the full numerical-photometric model, folded or shifted in the same way as the data.

Fig. 6. The detrended relative photometry of Kepler-9, folded on its orbital period, showing the full phase curve and the signal due to candidate KOI-377.03. For this, we adopt an ephemeris for KOI-377.03 of BJD 2454965.74 + $E \times 1.5925$. This light curve could be due to an astrophysical false positive, although the transit parameters and other considerations are consistent with this candidate being a coplanar third planet in the Kepler-9 system. Black vertical bars show phased photometry binned every ~ 15 min with error bars taken from the scatter of the data in each bin. Also shown is a model for the central transit of a planet with radius $\sim 1.5R_{\oplus}$.



but these could be affected by a young age (38). The heavy-element fractions of Kepler-9b and 9c are indeterminate (~ 0.1 to 0.5), but coreless (metal-free) models seem to be excluded.

KOI-377.03 might correspond to a super-Earth-size planet of radius $\sim 1.5R_{\oplus}$. With no additional information available for this planet, the upper mass limit of $7M_{\oplus}$ (where $M_{\oplus}$ is the mass of Earth) corresponds to the maximum mantle-stripping limit for a maximally iron-rich super-Earth (40). The lower mass limit is less clear: It could be as small as $1M_{\oplus}$ for a volatile-rich planet with a hot extended atmosphere; e.g., water steam (41, 42). The planet candidate is so close to its host star, comparable to CoRoT-7b (43), that its ~ 2200 K estimated surface temperature is very high, and volatile-rich solutions are less likely if the Kepler-9 planetary system is old and evaporation has been substantial. A volatile-poor rocky KOI 377.03 super-Earth with a Ganymede-like Fe/Si ratio would correspond to a planet mass of $\sim 3.5M_{\oplus}$ (44). Such a planet would have formed under volatile-rich conditions, only to lose all of its water due to evaporation.

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Supporting Online Material

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SOM Text
Figs. S1 to S4
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Piezo1 and Piezo2 Are Essential Components of Distinct Mechanically Activated Cation Channels

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Mechanical stimuli drive many physiological processes, including touch and pain sensation, hearing, and blood pressure regulation. Mechanically activated (MA) cation channel activities have been recorded in many cells, but the responsible molecules have not been identified. We characterized a rapidly adapting MA current in a mouse neuroblastoma cell line. Expression profiling and RNA interference knockdown of candidate genes identified *Piezo1* (*Fam38A*) to be required for MA currents in these cells. Piezo1 and related Piezo2 (*Fam38B*) are vertebrate multipass transmembrane proteins with homologs in invertebrates, plants, and protozoa. Overexpression of mouse Piezo1 or Piezo2 induced two kinetically distinct MA currents. Piezos are expressed in several tissues, and knockdown of Piezo2 in dorsal root ganglia neurons specifically reduced rapidly adapting MA currents. We propose that Piezos are components of MA cation channels.

Mechanotransduction, the conversion of mechanical force into biological signals, has crucial roles in physiology. In mammals, embryonic development, touch, pain, proprioception, hearing, adjustment of vascular tone and blood flow, flow sensing in kidney, lung growth and injury, bone and muscle homeostasis, as well as metastasis are all regulated by means of mechanotransduction (1, 2). In plants, mechanical

force strongly affects morphogenesis, for example, in lateral root formation (3). Unicellular organisms such as ciliates sense touch and change direction in response to a tactile stimulus (4). Mechanotransduction in vertebrate inner-ear hair cells is extremely rapid, implicating an ion channel directly activated by force (5). Indeed, calcium-permeable mechanically activated (MA) cationic currents have been described in various mecha-

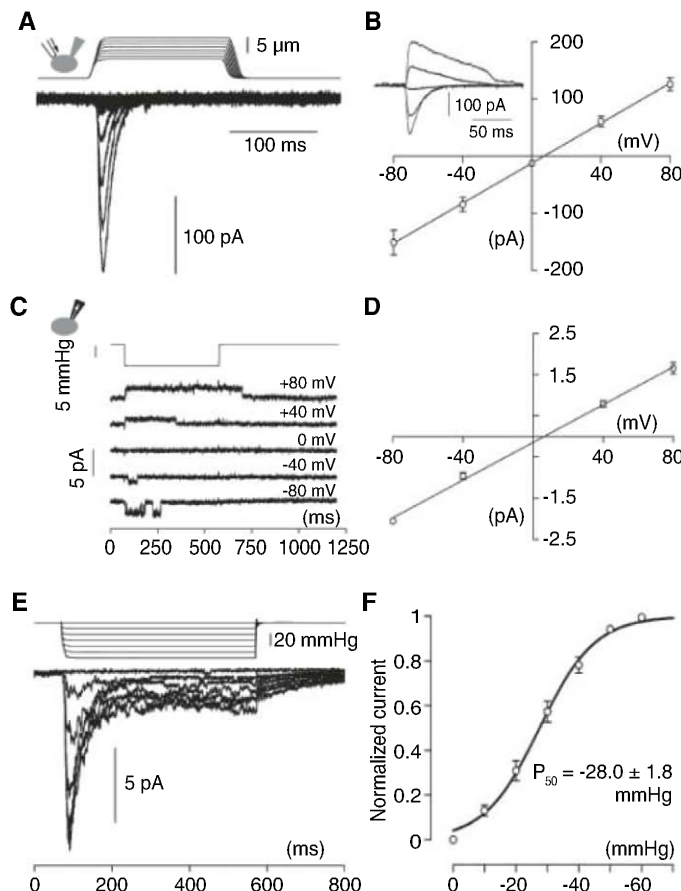
nonsensitive cells (2, 3, 6, 7). However, only few MA channels have been identified to date (1, 2), and definitive candidates in vertebrate mechanosensation has yet to emerge.

Neuro2A cells express MA currents. To identify proteins involved in mechanotransduction, we sought a cell line that expresses a MA current similar to those recorded from primary cells (8). We screened several mouse and rat cell lines (Neuro2A, C2C12, NIH/3T3, Min-6, 50B11, F11, and PC12), applying force to the cell surface via a piezo-electrically driven glass probe while patch-clamp recording in the whole-cell configuration with another pipette (6, 8, 9). The Neuro2A (N2A) mouse neuroblastoma cell line expressed the most consistent MA currents and showed relatively faster kinetics of adaptation (decreased activity in response to a sustained stimulus) as compared with that of other cell lines, such as C2C12s (Fig. 1, A and B, and fig. S1, A to D). Current-voltage relationships of N2A and C2C12 MA currents were linear between -80 and $+80$ mV with reversal potentials (E_{rev}) at $+6.6$ and $+6.7$ mV, respectively, and inward currents were suppressed with N-methyl-D-glucamine (NMDG)-chloride external solutions, suggesting cationic nonselective

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Fig. 1. MA currents in N2A cells. (A) Representative traces of MA inward currents expressed in N2A cells. Cells were subjected to a series of mechanical steps of $1\text{-}\mu\text{m}$ movements of a stimulation pipette (inset illustration, arrow) in the whole-cell patch configuration at a holding potential of -80 mV. (B) Average current-voltage relationships of MA currents in N2A cells ($n = 11$ cells). (Inset) Representative MA currents evoked at holding potentials ranging from -80 to $+40$ mV (applied 0.7 s before the mechanical step). (C) Single-channel currents (cell attached patch configuration) induced by means of negative pressure with a pipette (inset illustration, arrow) at holding potentials ranging from -80 mV to $+80$ mV in a N2A cell. (D) Average current-voltage relationships of stretch-activated single channels in N2A cells ($n = 4$ cells, mean $\pm$ SEM). Single-channel conductance was calculated from the slope of the linear regression line of each cell, giving $\gamma = 22.9 \pm 1.4$ pS (mean $\pm$ SEM). Single-channel amplitude was determined as the amplitude difference in Gaussian fits of full-trace histograms. (E) Representative currents (averaged traces) induced by means of negative pipette pressure (0 to -60 mmHg, $\Delta 10$ mmHg) in a N2A cell. (F) Normalized current-pressure relationship of stretch-activated currents at -80 mV fitted with a Boltzmann equation ($n = 21$ cells). P_{50} is the average value of P_{50} values from individual cells.



permeability (fig. S1E). We further characterized MA currents in N2A cells in response to suction of the membrane applied through the recording pipette in cell-attached mode (10). Negative pressure pulses evoked opening of endogenous channels (Fig. 1C), with a single-channel conductance

of 22.9 ± 1.4 pS and E_{rev} of $+6.2$ mV (Fig. 1D). Increasing the magnitude of pressure pulses induced larger and reversible currents (Fig. 1E). The current-pressure relationship is characterized by maximal opening at -60 mmHg, with a pressure for half-maximal activation (P_{50}) of $-28.0 \pm$

1.8 mmHg (Fig. 1F). These conductance and P_{50} values are similar to the properties of reported stretch-activated channels (11–13).

Piezo1 (Fam38A) is required for MA currents of N2A cells. To generate a list of candidate MA ion channels in N2A, we searched for

Fig. 2. Suppression of MA currents by means of Piezo1 (Fam38A) siRNA. **(A)** Average maximal amplitude of MA inward currents elicited at a holding potential of -80 mV in N2A cells transfected with scrambled siRNA (blue dot, $n = 56$ cells), Piezo1 (Fam38A) siRNA (red dot, $n = 20$ cells) or siRNA directed against other candidates tested (open symbols) (a list of candidates is available in table S1). For each candidate, the black circle and error bar represents the mean $\pm$ SEM, $n = 4$ to 27 cells each. The black line represents the average value of all cells tested ($n = 807$ cells), and the two blue dashed lines represent a fourfold decrease or increase of this value. **(B)** Average maximal amplitude of MA inward currents elicited at a holding potential of -80 mV in N2A cells transfected either with (blue) scrambled siRNA or (red) different Piezo1 (Fam38A) siRNAs. Smart-pool I is composed of four siRNAs, including siRNA 1, 2, and 3. $***P < 0.001$, Kruskal-Wallis test. (Inset) Representative traces of MA inward currents expressed in N2A cells transfected with (blue trace) scrambled siRNA or (red trace) Piezo1 (Fam38A) siRNA at a holding potential of -80 mV. **(C)** Representative currents (averaged traces) induced by means of negative pipette pressure (0 to -60 mmHg, $\Delta 10$ mmHg, cell attached) in a N2A cell transfected with (left) scrambled siRNA or (right) Piezo1 siRNA. Traces of current elicited by -60 mmHg are highlighted in blue and red. **(D)** Average maximal amplitude of stretch-activated currents elicited at a holding potential of -80 mV in N2A cells transfected with (blue) scrambled siRNA or (red) Piezo1 siRNA. Bars represent the mean $\pm$ SEM, and the number of cells tested is shown above the bars. $**P < 0.01$, unpaired t test with Welch's correction.

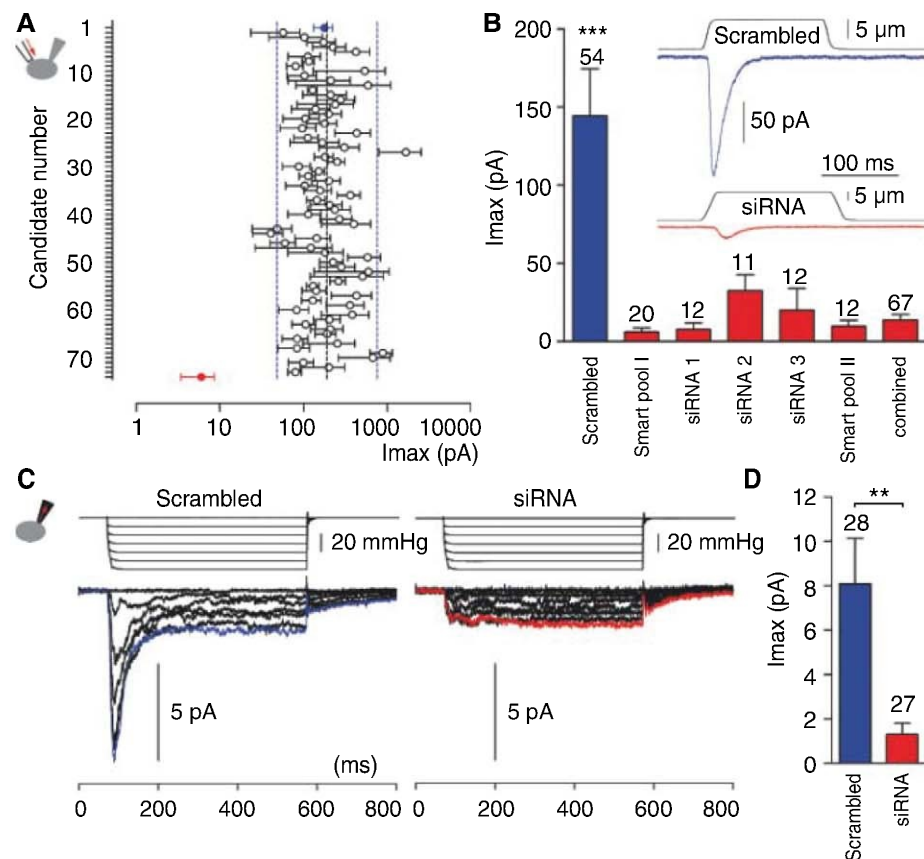
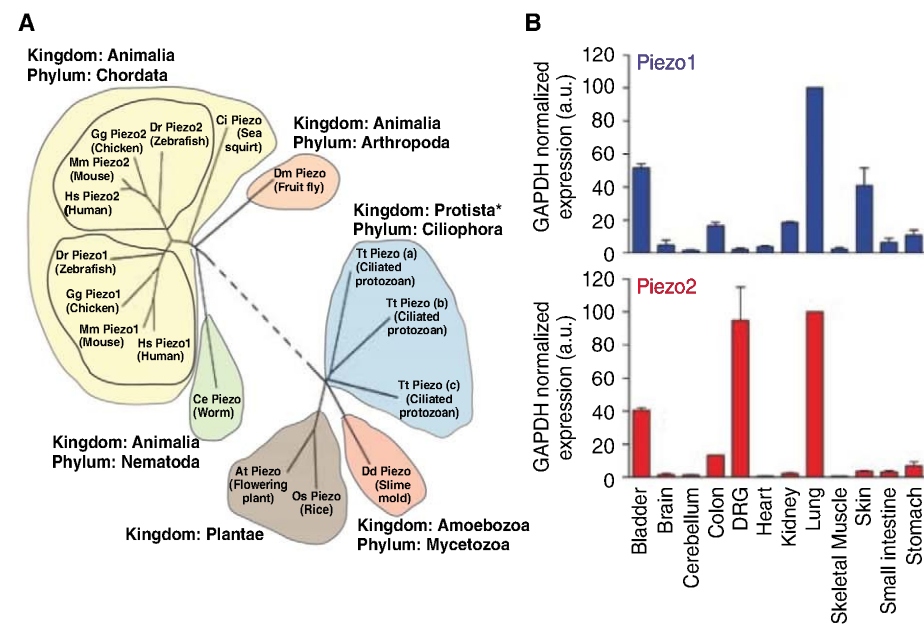


Fig. 3. Evolutionary conservation and expression profile of mouse Piezo1 and Piezo2. **(A)** Unrooted phylogenetic tree showing sequence relationship of different members of the Piezo family of proteins. The alignments were generated by using Megalign (DNASTAR, Madison, Wisconsin) and DrawTree programs. The dotted line represents an artificially extended line to accommodate fit. Hs, *Homo sapiens*; Mm, *Mouse musculus*; Gg, *Gallus gallus*; Dr, *Danio rerio*; Ci, *Ciona intestinalis*; Dm, *Drosophila melanogaster*; Ce, *Caenorhabditis elegans*; Dd, *Dictyostelium discoideum*; At, *Arabidopsis thaliana*; Os, *Oryza sativa*; and Tt, *Tetrahymena thermophila* [accession numbers are provided (25)]. Protista is referred to as a single kingdom but can be considered as a group of diverse phyla. **(B)** mRNA expression profiles of (top) Piezo1 and (bottom) Piezo2 determined by means of quantitative PCR from various adult mouse tissues. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the reference gene, and lung was used as the tissue calibrator by means of the $2^{-\Delta\Delta CT}$ method. Each bar is the mean $\pm$ SEM of the average of two separate experiments.



transcripts that are enriched in N2A cells using Affymetrix microarrays (Affymetrix, Santa Clara, California). We selected proteins predicted to span the membrane at least two times (a characteristic shared by all ion channels). We prioritized this list by picking either known cation channels or pro-

teins with unknown function. We tested each candidate (table S1) using small interfering RNA (siRNA) knockdown in N2A cells, measuring MA currents during piezo-driven pressure stimulation in the whole-cell mode. Knockdown of Fam38A (Family with sequence similarity 38) caused a

pronounced decrease of MA currents (Fig. 2A). Attenuation of MA currents was observed with multiple siRNAs directed against this gene (Fig. 2B). All the siRNAs tested decreased the abundance of the target transcripts as assayed with quantitative polymerase chain reaction (PCR) (fig. S2A). Given that Fam38A encodes a protein required for the expression of ion channels activated by pressure, we named this gene *Piezo1*, from the Greek "πίεση" (piesi), meaning pressure. To test whether depletion of *Piezo1* impairs general cell signaling or viability, we transfected N2A cells with TRPV1 cDNA (a capsaicin-activated cation channel) and either scrambled or *Piezo1* siRNA and observed no differences in capsaicin responses (fig. S2, B and C). We tested whether *Piezo1* was also required for N2A MA currents elicited through patch membrane stretch (Fig. 2C). MA currents were diminished in cells treated with siRNA against *Piezo1* (Fig. 2D).

Very little is known about mammalian *Piezo1* (KIAA0233, Fam38A, and Mib). Its expression is induced in senile plaque-associated astrocytes (14), and the protein has been suggested to be involved in integrin activation (15). Extracellular perfusion of cells with buffer lacking divalent ions and containing 5 mM EGTA for 30 to 60 min, which disrupts integrin function (16), did not suppress MA currents (fig. S2, D and E). Thus, it is unlikely that *Piezo1* siRNA blocks MA currents through integrin modulation. However, it is possible that mechanical activation of *Piezo1* could lead to integrin activation.

Piezos are large-transmembrane proteins conserved among various species. Many animal, plant, and other eukaryotic species contain a single *Piezo* (Fig. 3A). Vertebrates have two members, *Piezo1* (Fam38A) and *Piezo2* (Fam38B). However, the early chordate *Ciona* has a single member. Multiple *Piez*os are also present in the Ciliophora kingdom: *Tetrahymena thermophila* has three members; *Paramecium tetraurelia* has six. No clear homologs were identified in yeast or bacteria. The secondary structure and overall length of *Piezo* proteins are moderately conserved, and similarity to other proteins is minimal. As assayed with the Transmembrane Hidden Markov Model prediction program (TMHMM2) (CBS, Lyngby, Denmark), all have between 24 and 36 predicted transmembrane domains (with variability perhaps being due to inaccurate cDNA or transmembrane prediction). The predicted proteins contain 2100 to 4700 amino acids, and the transmembrane domains are located throughout the putative protein (fig. S3). *Piezo1* expression was observed in bladder, colon, kidney, lung, and skin (Fig. 3B). This pattern agrees with Northern blot expression analysis in rat (14). Bladder, colon, and lung undergo mechanotransduction related to visceral pain (17), and primary cilia in the kidney sense urinary flux (18). The relatively low amount of mRNA in dorsal root ganglia (DRGs) suggests that *Piezo1* may not account for MA currents observed there (8, 9, 19–22), but *Piezo1* was observed in the skin, which is another putative site

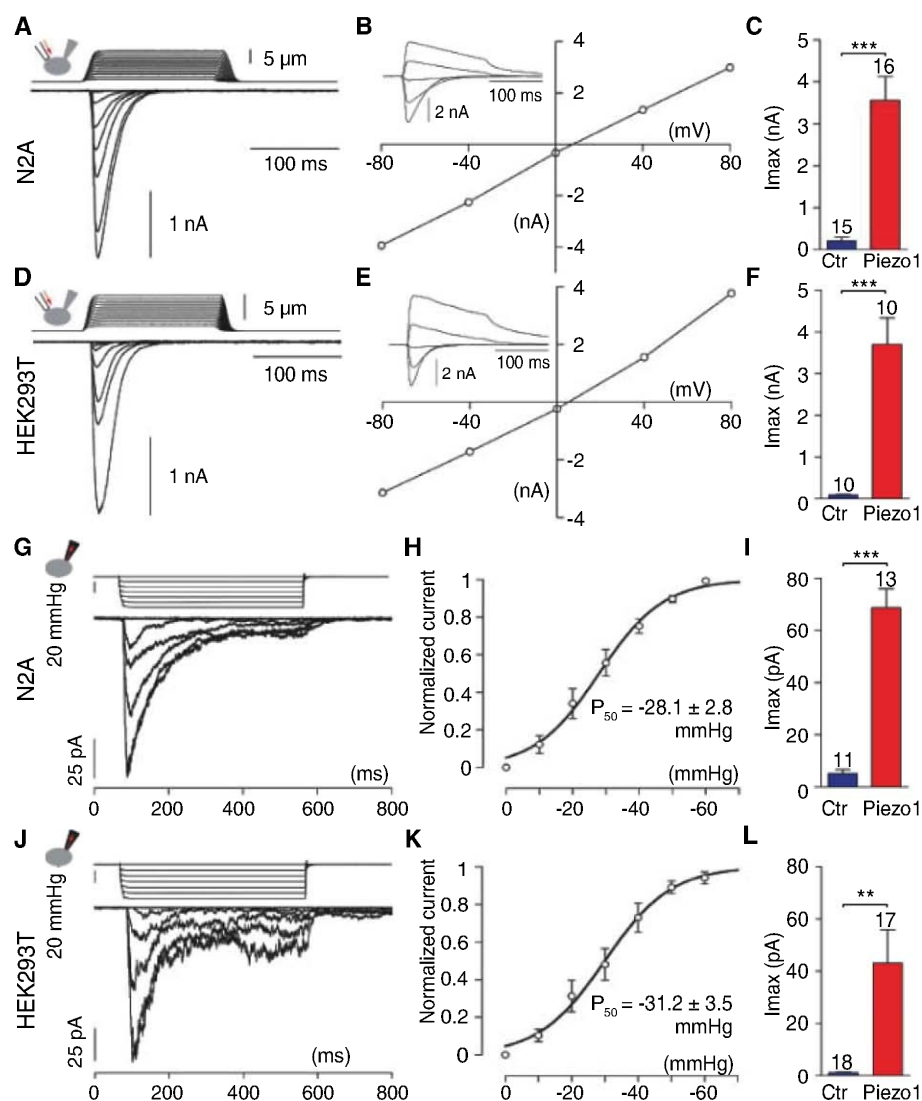


Fig. 4. Large MA currents from cells overexpressing *Piezo1*. [(A) to (F)] MA currents of *Piezo1*-expressing [(A) to (C)] N2A and [(D) to (F)] HEK293T cells recorded in the whole-cell configuration. [(A) and (D)] Representative traces of MA inward currents expressed in different cell types transfected with *Piezo1*. Cells were subjected to a series of mechanical steps in 1- μ m (A) or 0.5- μ m (D) increments by using glass probe stimulation and at a holding potential of -80 mV. [(B) and (E)] Representative current-voltage relationships of MA currents expressed in different cell types transfected with *Piezo1*. (Inset) MA currents evoked at holding potentials ranging from -80 to +40 mV. [(C) and (F)] Average maximal amplitude of MA inward currents elicited at a holding potential of -80 mV in (red) *Piezo1*-transfected or (blue) mock-transfected cells. Bars represent the mean $\pm$ SEM, and the number of cells tested is shown above the bars. *** $P < 0.001$, unpaired t test with Welch's correction. [(G) to (L)] Stretch-activated currents of mouse [(G) to (I)] *Piezo1*-expressing N2A and [(J) to (L)] HEK293T cells in cell-attached configuration. Representative averaged currents induced by means of negative pipette pressure (0 to -60 mmHg, Δ 10 mmHg) in (G) N2A and (J) HEK293T cells transfected with *Piezo1*. I_{max} normalized current-pressure relationship of stretch-activated currents elicited at -80 mV in (I) N2A and (L) HEK293T cells (blue) mock-transfected or (red) transfected with *Piezo1*. Bars represent the mean $\pm$ SEM, and the number of cells tested is shown above the bars. *** $P < 0.001$; ** $P < 0.01$, unpaired t test with Welch's correction.

of somatosensation. Piezo2 expression was observed in bladder, colon, and lung as well, but less abundant in kidney or skin. Strong expression of Piezo2 was observed in DRG sensory neurons, suggesting a potential role in somatosensory mechanotransduction.

Piezo1 induces MA currents in various cell types. We cloned full-length *Piezo1* from N2A cells into the pIRES2-enhanced green fluorescent protein (EGFP) vector. We recorded MA currents from GFP-positive cells in the whole-cell mode 12 to 48 hours after transfection. *Piezo1* but not mock-transfected cells showed large MA currents in N2A, human embryonic kidney (HEK) 293 T (Fig. 4, A to F), and C2C12 cell lines (fig. S4, A to C). In all cells overexpressing Piezo1, the MA current-voltage relationships were similar to those for endogenous N2A MA currents (Fig. 4, B and E, and figs. 1B and S4B), with $E_{rev} \sim +6$ mV. The threshold of activation and the time constant for inactivation of MA currents elicited in Piezo1-overexpressing cells was similar in all three cell lines tested (table S2). We characterized the ionic selectivity of MA currents

in cells overexpressing Piezo1. Substituting the nonpermeant cation NMDG in the extracellular bathing solution suppressed inward MA currents, demonstrating that this channel conducts cations (fig. S4, D and E). We further examined ionic selectivity by recording with CsCl-only internal solutions and various cations in the bath. Na^+ , K^+ , Ca^{2+} and Mg^{2+} all permeated, with a slight preference for Ca^{2+} (fig. S4, F to H). Moreover, 30 μ M of ruthenium red and gadolinium, which are known blockers of many cationic MA currents (9, 23), blocked $74.6 \pm 2.5\%$ ($n = 6$ cells) and $84.3 \pm 3.8\%$ ($n = 5$ cells) of Piezo1-induced MA current, respectively (fig. S4, I to K).

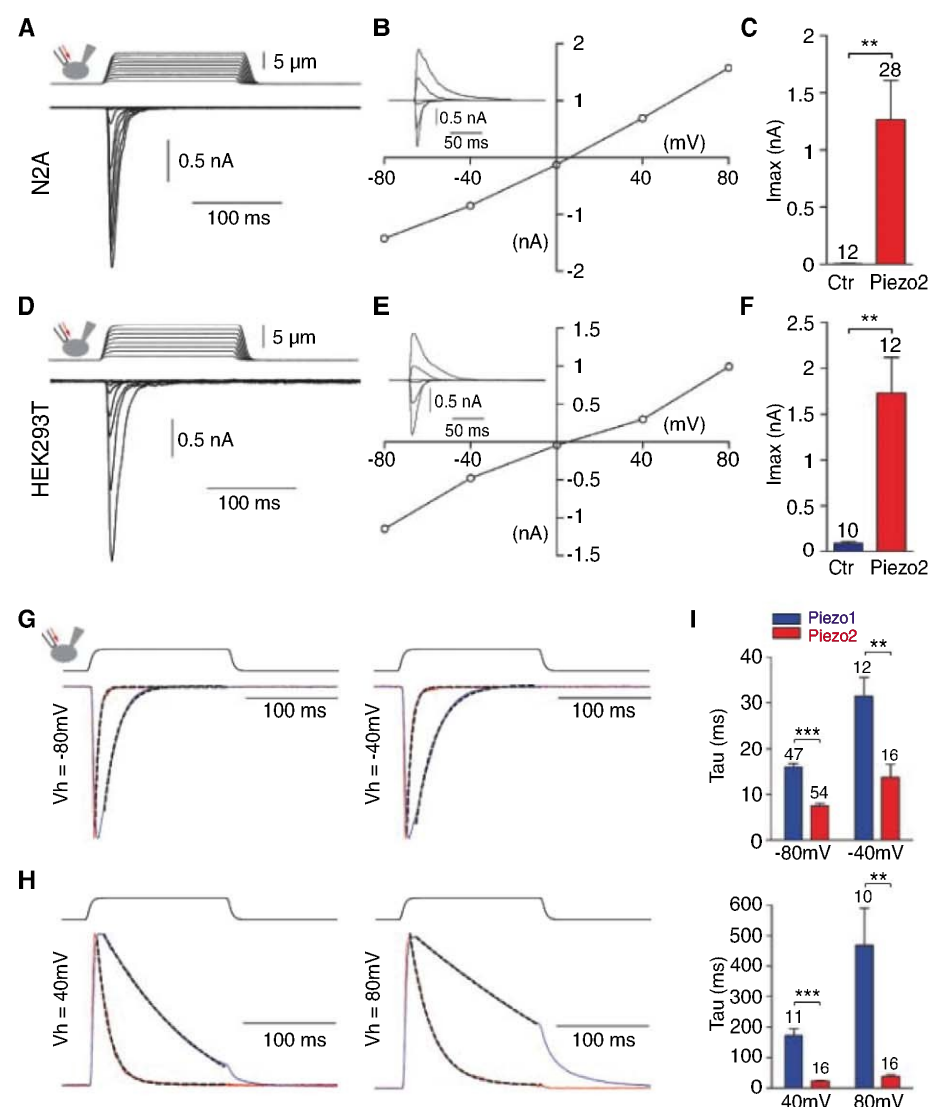
We used membrane stretch through the patch pipette in cell-attached mode to assay Piezo1-transfected cells (Fig. 4, G to L). Overexpression of Piezo1 in N2A and HEK293T cells gave rise to large currents elicited by -60 mmHg pressure pulses (Fig. 4, G and J). The current-pressure relationships in cells overexpressing Piezo1 and in endogenous N2A cells were similar, with P_{50} of -28.1 ± 2.8 and -31.2 ± 3.5 mmHg in N2A- and HEK293T-overexpressing cells, respectively

(Figs. 1F and 4, H and K). No channel activity similar to N2A endogenous MA channels was detected in HEK293T cells transfected with vector alone.

MA currents in cells overexpressing Piezo2. We cloned full-length *Piezo2* from DRG neurons. N2A and HEK293T cells transfected with Piezo2 and gene-encoding GFP showed large MA currents (Fig. 5, A to F). The N2A cells were also cotransfected with Piezo1 siRNA to suppress endogenous MA currents. The MA current-voltage relationship in Piezo2-expressing cells was linear between -80 and $+80$ mV (Fig. 5, B and E), with a E_{rev} of $+6.3 \pm 0.4$ mV ($n = 3$ cells) and $+8.7 \pm 1.5$ mV ($n = 7$ cells) in N2A and HEK293T cells, respectively. Piezo2-dependent currents were suppressed by NMDG (fig. S5, A and B), suggesting nonselective cationic conductance. Piezo2-dependent currents were inhibited by gadolinium and ruthenium red [$85.0 \pm 3.7\%$ ($n = 5$ cells) and $79.2 \pm 4.2\%$ ($n = 5$ cells), respectively] (fig. S5, C and D).

The inactivation kinetics of heterologously expressed Piezo2-induced MA currents were best

Fig. 5. Piezo2-dependent large MA currents kinetically distinct from Piezo1-induced currents. (A to F) MA currents of Piezo2-expressing [(A) to (C)] N2A and [(D) to (F)] HEK293T cells in whole-cell configuration. In N2A cells, Piezo2 or vector only were transfected with Piezo1 siRNA so as to suppress endogenous Piezo1-dependent MA currents. [(A) and (D)] Representative traces of MA inward currents expressed in different cell types transfected with Piezo2. Cells were subjected to a series of mechanical steps of $1\text{-}\mu\text{m}$ movements of a glass probe at a holding potential of -80 mV. [(B) and (E)] Representative current-voltage relationships of MA currents expressed in different cell types transfected with Piezo2. (Inset) MA currents evoked at holding potentials ranging from -80 to $+40$ mV. [(C) and (F)] Average maximal amplitude of MA inward currents elicited at a holding potential of -80 mV in (red) Piezo1-transfected or (blue) mock-transfected cells. (G and H) Representative traces of MA (G) inward or (H) outward currents expressed in cells transfected with (blue trace) Piezo1 or (red trace) Piezo2 at the specified holding potentials. Traces were normalized to the peak current, and dashed lines represent fits of inactivation with a mono-exponential equation. (I) Time-constant of inactivation of (blue) Piezo1 and (red) Piezo2 at negative (-80 and -40 mV, top) and positive (40 and 80 mV, bottom) holding potentials. Bars represent the mean $\pm$ SEM, and the numbers above bars are the number of cells. $**P < 0.01$; $***P < 0.001$, unpaired t test with Welch's correction.



fitted with a mono-exponential equation. The calculated time constants for inactivation (τ_{inac}) are relatively fast in both N2A (6.8 ± 0.7 ms, $n = 27$ cells) and HEK293T (7.3 ± 0.7 , $n = 11$ cells) cells when measured at -80 mV. Furthermore, the kinetics of inactivation of Piezo2-dependent MA currents were faster than Piezo1-dependent MA currents, both for inward (Fig. 5G) and outward (Fig. 5H) currents, and at all holding potentials tested (Fig. 5I). Therefore, Piezo1 and Piezo2 confer distinct channel properties.

Piezo1 is detected at the plasma membrane.

The results above suggest that Piezo1 and Piezo2 are components of mechanotransduction complexes and therefore should be present at the plasma membrane. Previous reports have shown expression of Fam38A (Piezo1) in the endoplasmic reticulum (14, 15). We generated a peptide anti-

body against mouse Piezo1. This antibody specifically recognized Piezo1-transfected HEK293T cells but not untransfected HEK293T cells (fig. S6A). In cells transfected with Piezo1 and TRPA1—an ion channel known to be expressed at the plasma membrane—we observed some overlap of Piezo1 staining with that of TRPA1 on the cell surface (24), although most Piezo1 and TRPA1 was present inside the cell (fig. S6B). Thus, Piezo1 protein can be localized at or near the plasma membrane. We could not detect expression of endogenous Piezo1 protein in N2A cells with this antibody.

Requirement of Piezo2 for rapidly adapting MA currents in DRG neurons. To characterize Piezo2 expression within the heterogeneous population of neurons and glial cells of the DRGs, we performed in situ hybridization on adult mouse DRG sections (Fig. 6A). We observed Piezo2

mRNA expression in 20% of DRG neurons (from 2391 total neurons) (25). Piezo2 was expressed in a subset of DRG neurons also expressing peripherin (60%) and neurofilament 200 (28%), which are markers present in mechanosensory neurons (26–29) (fig. S7). Some overlap with nociceptive marker TRPV1 (24%), further suggesting a potential role of Piezo2 in noxious mechanosensation. We used siRNA transfection to examine the role of Piezo2 in MA currents of DRG neurons. RNA interference (RNAi) on DRG neurons were validated on TRPA1, an ion channel expressed in DRG neurons and activated by mustard oil (MO) (30, 31) (fig. S8, A and B). siRNAs against Piezo2 were validated in N2A cells overexpressing Piezo2 cDNA (fig. S8C). We recorded whole-cell MA currents from DRG neurons transfected with GFP and either scrambled or Piezo2 siRNA ($n = 101$ neurons for scrambled and $n = 109$ neurons for Piezo2 siRNA). We grouped the recorded MA currents according to their inactivation kinetics (Fig. 6B) (8, 9, 19, 20, 22). We defined four different classes of neurons on the basis of τ_{inac} distribution in scrambled siRNA transfected cells (Fig. S8D): $\tau_{\text{inac}} < 10$ ms, $10 < \tau_{\text{inac}} < 30$, $\tau_{\text{inac}} > 30$ ms, and nonresponsive neurons. The proportion of neurons expressing MA currents with $\tau_{\text{inac}} < 10$ ms was specifically and significantly reduced in neurons transfected with Piezo2 siRNA as compared with that of neurons transfected with scrambled siRNA (Fig. 6C). 28.7% of scrambled siRNA-transfected neurons had $\tau_{\text{inac}} < 10$ ms, compared with 7.3% in Piezo2 siRNA-transfected neurons (Fig. 6D). Neurons with MA currents with slower kinetics (τ_{inac} between 10 and 30 ms and $\tau_{\text{inac}} > 30$ ms) were present at normal proportions in cells transfected with Piezo2 siRNA. We observed a trend toward increased numbers of mechanically insensitive neurons in populations expressing Piezo2 siRNA, as predicted if loss of Piezo2 converts rapidly adapting neurons into nonresponders. We also analyzed these RNAi data according to the degree of current inactivation during the 150-ms test pulse and came to similar conclusions (fig. S8E).

Discussion. We found that Piezo1 is required for MA currents in Neuro2A cells and that Piezo2 is required for a subset of MA currents in DRG neurons. Moreover, overexpressing Piezo1 or Piezo2 in three different cell types gave rise to a 17- to 300-fold increase in MA currents. We conclude that Piezos are both necessary and sufficient for the expression of a MA current in various cell types.

Piezo1 and Piezo2 sequences do not resemble those of other known ion channels or other protein classes. The large number of predicted transmembrane domains of Piezo1 and Piezo2 is reminiscent of the structure of voltage-activated sodium channels with 24 transmembrane domains, composed of a fourfold repeat of six-transmembrane units (32). However, pore-containing or repetitive domains have not been observed in Piezo proteins. It may be that Piezo proteins are non-conducting subunits of ion channels required for

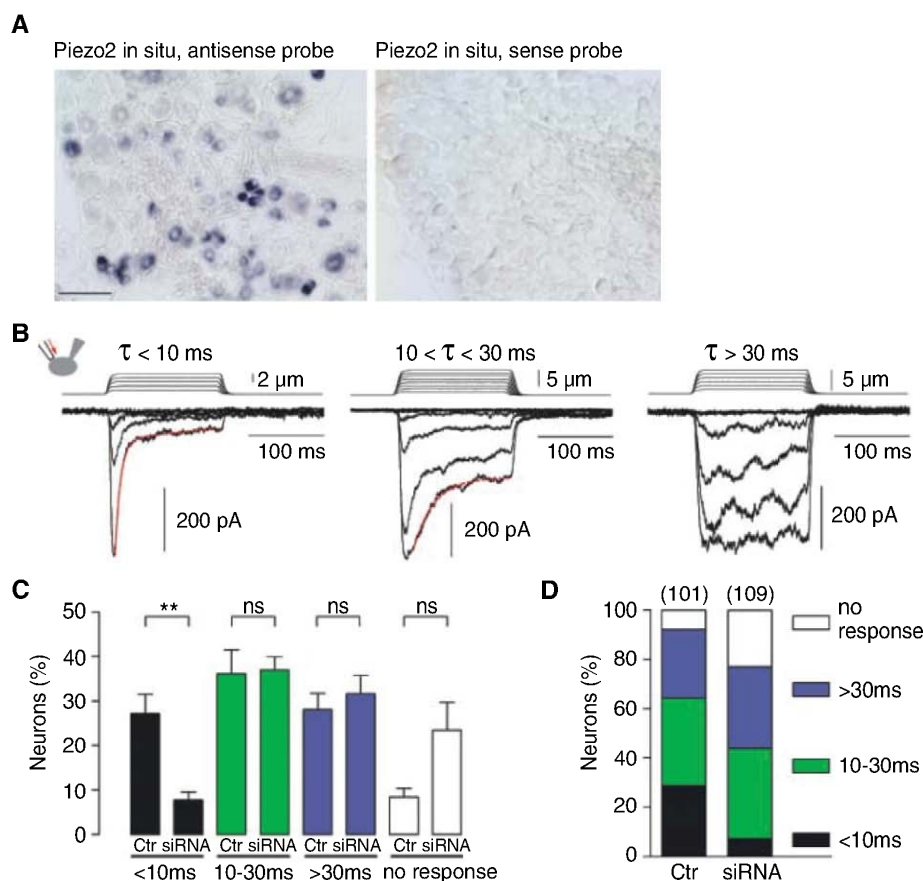


Fig. 6. Sensitivity of fast-inactivating MA currents in DRG neurons to depletion of Piezo2. **(A)** Representative images of colorimetric in situ hybridization for Piezo2 in DRG neurons by using (left) antisense and (right) sense probes. **(B)** Representative traces of three typical MA inward currents expressed in DRG neurons are characterized by distinct inactivation kinetics. Neurons were subjected to a series of mechanical steps in 1- μ m increments at a holding potential of -80 mV. Current inactivation was fitted with (left) a bi-exponential equation, giving fast time-constant (τ) of 7.3 ms and slow time-constant > 100 ms, or (middle) a mono-exponential equation, giving a time constant of 27 ms. Some currents with $\tau > 30$ ms are too slow to be efficiently fitted during the (right) 150-ms step stimulation. **(C and D)** Frequency histograms indicating the proportion of neurons transfected with scrambled siRNA (Ctrl) or Piezo2 siRNA (siRNA) that respond to mechanical stimulation, with MA currents characterized by their inactivation kinetic. Bars represent the mean $\pm$ SEM of (B) the proportion of neurons from seven separate experiments ($n = 12$ to 19 neurons per condition and per experiment) or (C) the proportion from all neurons pooled from all seven experiments; the numbers above bars in (C) represent the number of neurons. $**P < 0.01$; ns, not significantly different; unpaired t test.

proper expression or for modulating channel properties, similar to β subunits of voltage-gated channels (32) or SUR subunits of adenosine 5'-triphosphate-sensitive K⁺ channels (33). In this case, all the cell types used here would have to express an inactive conducting subunit of an MA channel that requires Piezos to function. Alternatively, Piezo proteins may define a distinct class of ion channels, akin to Orai1, which lacks sequence homology to other channels (34). Piezo1 is also found in the endoplasmic reticulum (14, 15), so Piezos may act at both the plasma membrane and in intracellular compartments.

We described a role of Piezo2 in rapidly adapting MA currents in somatosensory neurons. Thus, Piezo2 has potential roles in touch and pain sensation (35, 36). Piezo1 and Piezo2 are expressed in various tissues, and their homologs are present throughout animals, plants, and protozoa, raising the possibility that Piezo proteins have a broad role in mechanotransduction.

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37. The authors gratefully acknowledge H. Hu for help in cloning Piezo2, J. Walker for contributing to microarray experiments, S. Batalov for help in bioinformatics analysis, K. Spencer for help with imaging, and N. Hong and U. Müller for comments on the manuscript and helpful discussions. This work was supported by grants from NIH (DE016927 and NS046303) and the Novartis Research Foundation. B.C. is the recipient of an American Heart Association postdoctoral fellowship; M.S. is the recipient of a German Academic Exchange Service (DAAD, D/07/41089) fellowship. A provisional patent has been filed by TSRI and GNF that claims methods of screening for molecules that modulate Piezo-dependent ion channel activities. The Piezo1 and Piezo2 sequences used in the paper have been deposited in GenBank under accession numbers HQ215520 and HQ215521, respectively.

Supporting Online Material

www.sciencemag.org/cgi/content/full/science.1193270/DC1
Materials and Methods

Figs. S1 to S8

Tables S1 and S2

References

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REPORTS

Universal Dynamical Decoupling of a Single Solid-State Spin from a Spin Bath

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Controlling the interaction of a single quantum system with its environment is a fundamental challenge in quantum science and technology. We strongly suppressed the coupling of a single spin in diamond with the surrounding spin bath by using double-axis dynamical decoupling. The coherence was preserved for arbitrary quantum states, as verified by quantum process tomography. The resulting coherence time enhancement followed a general scaling with the number of decoupling pulses. No limit was observed for the decoupling action up to 136 pulses, for which the coherence time was enhanced more than 25 times compared to that obtained with spin echo. These results uncover a new regime for experimental quantum science and allow us to overcome a major hurdle for implementing quantum information protocols.

In the past decade, manipulation and measurement of single quantum systems in the solid state have been achieved (1, 2). This control has promising applications in quantum information processing (3, 4), quantum commu-

nication (5), metrology (6), and ultrasensitive magnetometry (7, 8). However, uncontrolled interactions with the surroundings inevitably lead to decoherence of the quantum states (9) and pose a major hurdle for realizing these technologies. Therefore, the key challenge in current experimental quantum science is to protect individual quantum states from decoherence by their solid-state environment.

If a quantum system can be controlled with high fidelity, dynamical decoupling can be ex-

ploited to efficiently mitigate the interactions with the environment (10–12). By reversing the evolution of the quantum system at specific times with control pulses, the effect of the environment accumulated before the pulse is canceled during the evolution after the pulse. When viewed at the end of the control cycle, the quantum system will appear as an isolated system that is decoupled from its environment. Thanks to recent progress in quantum control speed and precision (13, 14), we can now unlock the full power of dynamical decoupling at the level of a single spin.

We focused on electron spins of single nitrogen-vacancy (NV) defect centers in diamond coupled to a spin bath (Fig. 1A). NV center spins can be optically imaged, initialized, and read out, as well as coherently controlled at room temperature (Fig. 1B). These favorable properties have been exploited to gain deeper insight into spin decoherence (15, 16), as well as for demonstrating basic quantum information protocols at room temperature (17, 18).

We used nanosecond microwave pulses to manipulate single NV spins. To raise the fidelity of our control to the required level for efficient decoupling, we fabricated on-chip coplanar waveguide (CPW) transmission lines using electron beam lithography (Fig. 1A). The high bandwidth of the CPW (13) combined with efficient suppression of reflections and fine-tuned pulse calibration (14) allows fast and precise manipulation of

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the NV spin (Fig. 1B), leading to process fidelities of 99% for the basic control pulses needed for dynamical decoupling (14).

The coherent dynamics of an NV spin are strongly influenced by the coupling to neighboring spins (the spin bath) (15, 16). Because such spin environments are common in the solid state, our results are directly relevant for other solid-state quantum bits such as spins in quantum dots (19, 20) and donors in silicon (4, 21). For the NV centers studied here, the bath is composed of electron spins localized on nitrogen impurity atoms. Resonant interactions (flip-flops) between the bath spins and the NV spin are suppressed because of a large energy mismatch (16). Therefore, the impact of the spin bath on the NV spin is limited to dephasing and can be described as a random magnetic field $B(t)$ that is directed along the NV's quantization axis. The value of $B(t)$ is determined by the state of the environment. We modeled the bath field $B(t)$ by an Ornstein-Uhlenbeck process with the correlation function $C(t) = \langle B(0)B(t) \rangle = b^2 \exp(-|t|/\tau_c)$, where b is the coupling strength of the bath to the spin and τ_c is the correlation time of the bath, which measures the rate of flip-flops between the bath spins due to the intrabath dipolar coupling (14, 22).

The values of the parameters describing the bath field were extracted from experiments. The bath-induced dephasing during free evolution had a Gaussian envelope $S(t) = \exp(-b^2 t^2/2)$, which yielded the value for b (14); we found $b = (3.6 \pm 0.1) \mu\text{s}^{-1}$ for NV1 (Fig. 1C), and $b = (2.6 \pm 0.1) \mu\text{s}^{-1}$ for NV2 (14). The quasi-static dephasing could be undone with a spin echo (SE) technique (Fig. 2A), revealing the much slower decay of spin coherence caused by the dynamics of the spin bath. The spin echo signal decayed as $SE(t) = \exp[-(t/T_2)^2]$, characteristic for a slowly fluctuating spin bath with $\tau_c = T_2^3 b^2/12 \gg 1/b$ (22). The values we found for τ_c , $(25 \pm 3) \mu\text{s}$ for NV1 [$T_2 = (2.8 \pm 0.1) \mu\text{s}$] and $(23 \pm 3) \mu\text{s}$ for NV2 [$T_2 = (3.5 \pm 0.2) \mu\text{s}$], confirmed this. The spin echo decay time T_2 is often considered as the coherence or memory time of the system. We took T_2 as the starting point and demonstrated that the coherence time could be markedly prolonged by dynamically decoupling the spin from the surrounding spin bath.

We first explored the potential of dynamical decoupling by extending the SE pulse sequence to periodic repetitions of the Carr-Purcell-Meiboom-Gill (CPMG) cycle (Fig. 2A). The decoupling performance was characterized by measuring the state fidelity $F_s = \langle \psi_f | \rho_m | \psi_f \rangle$, where $|\psi_f\rangle$ is the expected (ideal) state after applying the sequence and ρ_m the measured density matrix of the actual state. Although the coherence had vanished after 4 μs for the SE case, we observed that the eight-pulse CPMG sequence preserved the coherence almost completely during this same time.

The optimal decoupling sequence for a quantum system depends on the coupling to its environment and the dynamics within the environment itself. In (23), nonperiodic interpulse spacing,

now called the UDD sequence, was found to achieve a strong improvement in decoupling efficiency over periodic pulse spacing in the case of environmental noise spectra with a hard cut-

off; this was experimentally verified in (24, 25). Recent theory (26, 27), however, suggests that periodic, CPMG-like pulse spacing is ideal for decoupling from an environment with a soft cut-

Fig. 1. Quantum control of a single spin in diamond. **(A)** Left: A nitrogen-vacancy defect is formed by a single substitutional nitrogen (^{14}N) atom and an adjacent vacancy (V). The NV electron spin (orange arrow) is coupled to the host ^{14}N nuclear spin (blue arrow) through the hyperfine interaction. Middle: The NV center is surrounded by a bath of electron spins located at sites of substitutional nitrogen atoms in the diamond lattice (16). Right: Confocal photoluminescence scan of a section of the device, where the golden regions are part of the on-chip coplanar waveguide (CPW) used for applying quantum control pulses and NV centers appear as bright spots in between the conductors of the CPW. **(B)** Energy level diagrams of the NV center electron spin (left) and the electron spins in the bath (right). An applied magnetic field splits the NV spin triplet electronic ground state; the effective two-level system used here is formed by the spin sublevels $m_s = 0$ (labeled $|0\rangle$) and $m_s = -1$ (labeled $|1\rangle$) (14). **(C)** Coherent driven oscillations of NV1. For the pulsed experiments, the same Rabi frequency is used (14). **(D)** Decay during free evolution of NV1 probed using Ramsey interference. Solid line is a fit (14). The fast oscillating component is due to a detuning of the driving field of 15 MHz with respect to the spin transition, whereas the beating is caused by the hyperfine interaction with the host nuclear spin.

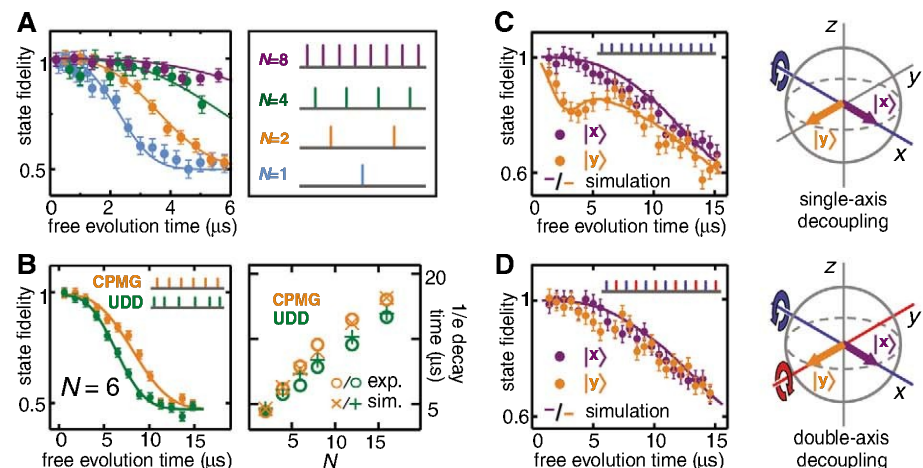
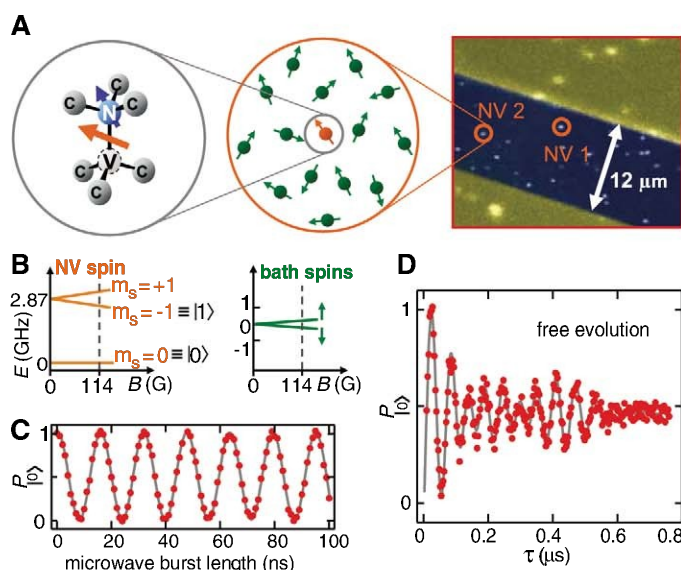


Fig. 2. Optimized dynamical decoupling of NV1. **(A)** Left: State fidelities for CPMG decoupling sequence applied to NV1. The blue curve is a spin echo measurement. High state fidelity is recovered for increasing number of pulses N . Solid lines are fits to $\sim \exp[-(t/T_{\text{coh}})^2]$. Right: Vertical lines indicate the location of π -pulses. **(B)** Comparison of decoupling with CPMG (orange) and UDD (green) for $N = 6$ pulses. The solid lines are fits to $\sim \exp[-(t/T_{\text{coh}})^2]$. The right panel shows the 1/e decay times from fits to data and to simulations (14). The same color scheme applies. **(C)** Single-axis decoupling for different input states, showing state-selective decoupling for the CPMG sequence with $N = 12$ operations (shown in the upper right). Bloch sphere on the right shows input states and the decoupling axis. Solid lines are numerical simulations incorporating the experimental pulse errors (14). **(D)** Double-axis decoupling, with XY4 sequence with $N = 12$, showing excellent decoupling for both input states. Pulse timings are the same as for CPMG but with the decoupling axis alternating between X and Y, as shown on the right. The simulations for $|x\rangle$ and $|y\rangle$ yield practically the same curve and therefore appear as one.

off. We investigated the efficiency of these different protocols in decoupling a single spin from a spin bath environment (Fig. 2B) and observed that CPMG outperformed UDD for all numbers of pulses investigated in both simulations and experiments (Fig. 2B, right panel). These findings are in agreement with our model of a Lorentzian bath noise spectrum, which exhibits a soft cut-off (14).

For applications in quantum information processing, it is essential that the decoupling protocol is universal, meaning that it can preserve coherence for arbitrary quantum states. Because pulse errors can severely degrade the coherence, universal decoupling requires robustness to pulse errors for all possible quantum states. In contrast, protocols that use single-axis decoupling such as CPMG optimally preserve only a limited range of quantum states, whereas for other quantum states the pulse errors accumulate rapidly with increasing number of control pulses. In Fig. 2C, we demonstrated this experimentally by comparing the decay curves of superposition states aligned ($|x\rangle$) and perpendicular ($|y\rangle$) to the CPMG decoupling axis. Even though the fidelity of the single-pulse control was very high (14), the remaining small errors caused a significant loss

of decoupling fidelity for state $|y\rangle$ when the number of operations was increased to 12 pulses; this effect was accurately reproduced by simulations (Fig. 2C) (14).

The use of sequences containing decoupling pulses over two axes, such as XY4 (Fig. 2D) (28), avoids this selective robustness to pulse errors and can compensate certain systematic pulse errors and coherent resonant perturbations without increasing control overhead. We found that XY4 could indeed preserve both quantum states $|x\rangle$ and $|y\rangle$ (Fig. 2D).

We studied the decoupling performance in more detail with the use of quantum process tomography (QPT), which allows for a complete characterization of any quantum process (29). Figure 3 shows the experimental QPT results for XY4 with $N = 8$ operations, at different free evolution times. For a free evolution time of 4.4 μs , much longer than T_2 , the measured process matrix χ is in excellent agreement with the ideal process of identity that is expected for perfect universal decoupling.

By taking snapshots of the process for different free evolution times, we monitored how decoherence affects the quantum states. We observed that after $t = 10 \mu\text{s}$, the process element corresponding to identity had decreased, whereas the $\sigma_z\sigma_z$ element had grown. After 20 μs , these elements had approximately equal amplitudes. This behavior is characteristic for pure, off-diagonal dephasing (29) and is consistent with our model of the environment, in which the magnetic dipolar coupling with the bath leads to phase randomization. The independently measured energy relaxation time $T_1 > 1 \text{ ms}$ (14) confirmed that longitudinal decay is not relevant in this regime.

Finally, we investigated how the coherence time scales with the number of control pulses. A detailed theoretical analysis showed that for N perfect pulses, the decoupling fidelity decayed as $F(t) = \exp[-AN^2/(2N\tau_c)^3]$, where the total free

evolution time $t = 2N\tau$ and 2τ is the interpulse distance (14). For the XY4 sequence, we found that $A = (2/3)b^2\tau_c^2$ for both large and small N . The theory predicts two interesting features: (i) The decay follows the universal form $\exp[-(t/T_{\text{coh}})^3]$ for all N , and (ii) the $1/e$ decay time scales as $T_{\text{coh}}(N) = T_2N^{2/3}$.

In Fig. 4A, we show XY4 decoupling for $N = 4, 16$, and 72, as well as the spin echo for comparison. These data indicate that the $1/e$ decay time indeed scales with the number of pulses. For a thorough comparison with the theory, we renormalized the time axis to $T_2N^{2/3}$ (Fig. 4B). We found that all data collapse onto a single curve in line with the prediction. Then, we plotted the $1/e$ decay time of coherence of NV1 and NV2 and fit this to the expected scaling law. The data of both NV centers showed excellent agreement with the theory over a range in N spanning two orders of magnitude. For the longest sequence applied (136 pulses), the coherence time was increased by a factor of 26.

Is there a limit to the coherence enhancement that can be achieved with dynamical decoupling? Our results demonstrate that we can prolong the spin coherence beyond the bath correlation time τ_c . Also, the nuclear spin bath, which would affect the NV dynamics on a 5- μs time scale for the magnetic field used here (15), is efficiently decoupled from the NV spin. Indeed, the theory indicates no fundamental limit to the coherence time. In practice, the decoupling efficiency will be limited by the minimum interpulse delay (on the order of the pulse widths) and the longitudinal relaxation time.

Because the spin bath environment is common to solid-state quantum bits, our findings can be transferred to other promising systems such as spins in quantum dots (3, 19, 20) and donors in silicon (4, 21). Furthermore, the performance of spin-based magnetometers can greatly benefit from this work, because the magnetic field sensitivity scales with the coherence time (7, 8). Fi-

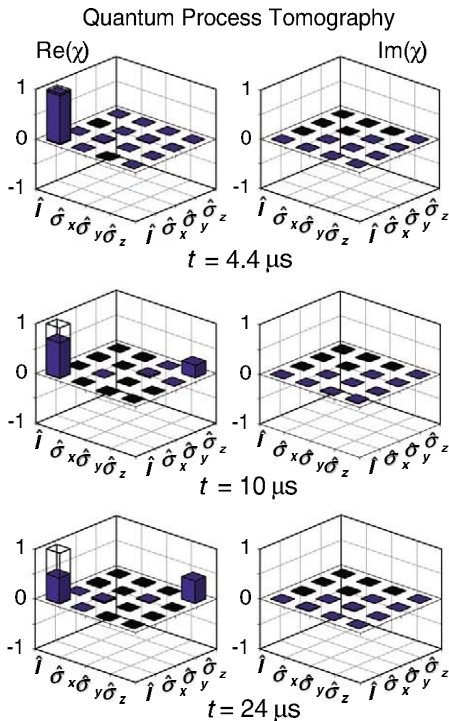
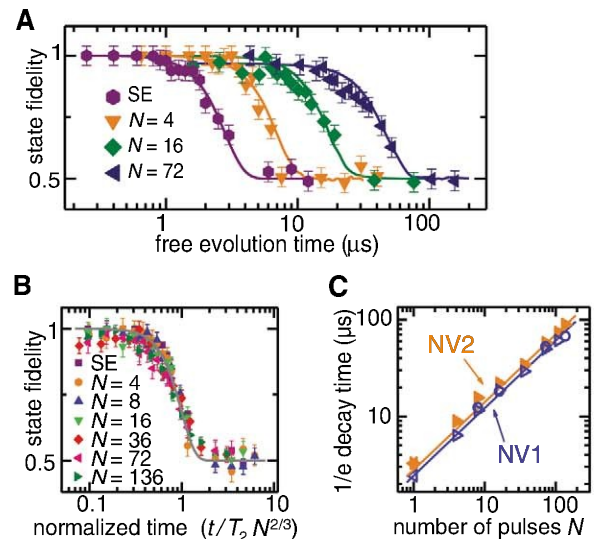


Fig. 3. Universal decoupling demonstrated with quantum process tomography. QPT is performed at free evolution times of 4.4, 10, and 24 μs for XY4 with $N = 8$ (see Fig. S2B). At $t = 4.4 \mu\text{s}$, the measured process matrix nearly equals the identity process matrix χ (fidelity of 0.96 ± 0.02), indicating close-to-perfect quantum state protection. At longer free evolution times, the process changes into pure dephasing in accordance with our model of the spin bath.

Fig. 4. Scaling of the coherence enhancement with number of control pulses. (A) Decoupling for different number of control pulses N . Increasing N extends the coherence to longer times. Solid lines are simulations (14). (B) Data rescaled to the normalized time axis $t/(T_2N^{2/3})$. (C) Coherence $1/e$ decay time (T_{coh}) plotted as a function of the number of control pulses for NV1 and NV2. Solid lines are fits to $T_{\text{coh}}(N) = T_2N^{2/3}$ with T_2 as free parameter.



nally, dynamical decoupling can be applied to protect entangled states, which are at the heart of quantum information science.

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Multiple Exciton Collection in a Sensitized Photovoltaic System

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Multiple exciton generation, the creation of two electron-hole pairs from one high-energy photon, is well established in bulk semiconductors, but assessments of the efficiency of this effect remain controversial in quantum-confined systems like semiconductor nanocrystals. We used a photoelectrochemical system composed of PbS nanocrystals chemically bound to TiO₂ single crystals to demonstrate the collection of photocurrents with quantum yields greater than one electron per photon. The strong electronic coupling and favorable energy level alignment between PbS nanocrystals and bulk TiO₂ facilitate extraction of multiple excitons more quickly than they recombine, as well as collection of hot electrons from higher quantum dot excited states. Our results have implications for increasing the efficiency of photovoltaic devices by avoiding losses resulting from the thermalization of photogenerated carriers.

The urgent need for massively scalable carbon-free energy sources has focused attention on both increasing the efficiency and decreasing the cost of photovoltaic cells. When electrons are excited by photons with energy (E_{hv} , where h is Planck's constant and ν is photon frequency) in excess of a semiconductor band gap, they tend to rapidly thermally relax to the conduction band edge; in this context, Shockley and Queisser calculated the maximum solar to electrical energy conversion efficiency for an optimal single band gap (E_g) semiconductor absorber to be about 31% (1). Third-generation solar cells have their basis in concepts that can potentially circumvent the so-called Shockley-Queisser limit (2). One such mechanism currently under active investigation (3–5) is to convert the excess energy of incident photons with $E_{hv} \geq 2E_g$ into additional free carriers in the material. An ideal material would produce two carriers per photon beginning at $E_{hv} = 2E_g$ and additional

carriers for photons with energies equal to multiples of E_g [i.e., for $E_{hv} = 4E_g$, four carriers are generated per photon; however, 94% of the maximum gain in power conversion efficiency would be produced with just two carriers per photon (6)]. This process is known as carrier multiplication via impact ionization in bulk semiconductors but is

quite inefficient because it usually requires E_{hv} to be much greater than $2E_g$ to generate an additional carrier per incident photon. However, there have been suggestions (7, 8) that the process could be more efficient in semiconductor nanocrystals or quantum dots (QDs) because of the electronic structure associated with carrier confinement in three dimensions. In QDs, the process is known as multiple exciton generation (MEG) because the carriers are not free but instead are correlated as a result of confinement. Optical measurements of various nanomaterial systems, including colloidal QD solutions (9–17), QD thin films (18), and single-walled carbon nanotubes (SWCNT) (19), have identified signatures of MEG, but the generation efficiency in nanomaterials relative to bulk materials is still under discussion (20–22).

Despite numerous reports of optical detection of MEG in QDs, multiple exciton collection (MEC) from QDs, converting absorbed photons into photocurrents with quantum yields greater than one, has not yet been observed in a photovoltaic device. Recent reports measured MEG photocurrent in individual SWCNT photodiodes operating at low temperatures (23), separation of

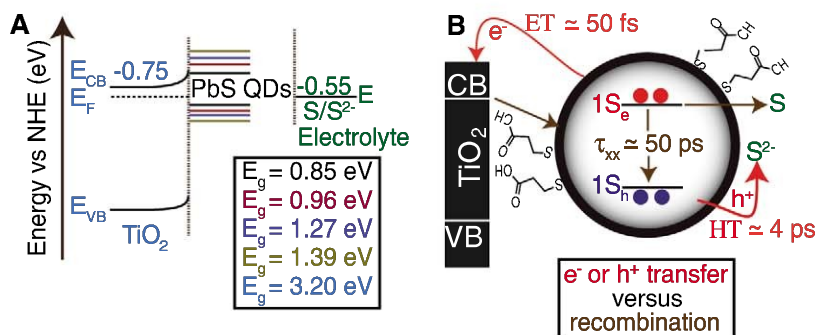


Fig. 1. Band energy diagram indicating the relevant energy levels and kinetic processes that describe PbS QD ET and HT into the TiO₂ conduction band and the sulfide/polysulfide electrolyte, respectively. (A) Energy-level alignment of the TiO₂ conduction band (35) with various sized PbS QDs and the S/S²⁻ redox couple at pH 13. (Inset) The band gap energies of TiO₂ and the QDs used in this study. (B) Representation of a QD adsorbed on a TiO₂ single crystal and the approximate time scales for efficient ET and HT compared with the biexciton lifetime (τ_{xx}), as well as other possible recombination pathways. $1S_e$ and $1S_h$ refer to the first excited electron and hole state, respectively. Red and brown arrows indicate the favorable processes and the possible recombination pathways, respectively.

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multiexcitons generated by multiphoton absorption in colloidal CdSe QDs with physisorbed electron acceptors (24), and a photodetector made with PbS QDs that showed enhanced photoconductivity at higher photon energies attributed to MEG (25). High short-circuit photocurrents have been achieved in photovoltaic devices consisting of several-hundred-nm-thick layers of PbSe (26) or PbS (27, 28) QDs, but quantum yields greater than unity have not been confirmed.

The dye-sensitized solar cell, a subject of intense research since its invention in 1991 (29), is a photoelectrochemical photovoltaic device that has the potential to be cost-effectively mass-produced. The most common manifestation of this device consists of a thin film of inexpensive nanocrystalline titanium dioxide that acts as both a charge-transporting substrate and a high-surface-area scaffold for attaching visible light-absorbing dye molecules (sensitizers) that inject photoexcited electrons into the TiO₂ conduction band. Recently QDs have been investigated as sensitizers because of their potential for enhanced stability compared with conventional dyes, as well as high light absorption cross sections that can be tuned to cover a large fraction of the solar spectrum simply by varying the particle size (30, 31). Despite such beneficial attributes, quantum dot-sensitized solar cells (QDSSCs) have not achieved efficiencies or stabilities competitive with conventional dye-sensitized solar cells. One reason for this is that the surface chemistry for the chemical attachment of the QDs to the TiO₂ surface was not well understood or controlled. In several of our recent studies, we used single crystals of both anatase and rutile forms of TiO₂ as simple model systems to evaluate the influence of different QD attachment procedures on the electronic coupling of CdSe QDs and CdSe/ZnS core/shell QDs to the TiO₂ surface by measuring the photocurrent yields due to electron transfer from photoexcited QDs into TiO₂ (32, 33). We used a surface chemistry strategy whereby short-chain, bifunctional passivating ligands such as 3-mercaptopropionic acid (MPA) stabilize the QDs in water while chemically binding the nanocrystals to the TiO₂ surface via thiolate and carboxylic acid moieties, respectively (34). Atomic force microscopy (AFM) confirmed that our surface chemistry strategy reproducibly resulted in a single layer of QDs covalently bound to the atomically flat single-crystal substrates with no three-dimensional QD clusters.

The minimum photon energy for observing MEG in CdSe or CdSe/ZnS QDs would be twice the bulk band gap (>3.4 eV). Considering that quantum confinement increases the band gap compared with the bulk, we decided to shift our focus to PbS, with a considerably lower bulk band gap value of 0.37 to 0.41 eV at 300 K (35). PbS QDs are readily synthesized with band gap energies ranging from 0.5 to 2.0 eV, making it possible to measure sensitized photocurrents associated with MEG by using photons sufficiently low in energy to preclude direct excitation of the TiO₂ band gap (3.0 eV for rutile and 3.2 eV for anatase).

The kinetically controlled pathways for photogenerated electrons and holes in a QDSSC are depicted in Fig. 1. Efficient production of sensitized photocurrents requires the energy of the QD excited state to be higher (more negative on the electrochemical scale) than the conduction band energy of the semiconductor substrate and well electronically coupled to the conduction band states of the semiconductor (Fig. 1A). After electron transfer (ET), the photooxidized QD is reduced by hole transfer (HT) to a redox species in solution with a reduction potential more negative than the ground state of the QD. In addition to the energetic constraints for efficient ET or HT, various recombination processes such as ET from the TiO₂ conduction band to the QDs (or electrolyte), as well as relaxation of the photoexcited electron to the QD ground state, compete with the forward processes (Fig. 1B). Therefore the ratio of rates of the forward (ET or HT) to the reverse (recombination) processes must be high enough to ensure that photocurrent generation is kinetically favored. Aside from the general recombination mechanisms inherent in QDSSCs, MEC requires very fast electron injection in order to outpace exciton-exciton annihilation. The measured fast electron injection times of <1 ns (36) and 50 fs (37) from photoexcited PbS and PbSe QDs, respectively, into TiO₂, as well as a 4-ps hole transfer time from PbS QDs to a solid-state organic hole acceptor (38), suggest that MEC can occur on a faster time scale than the 50-ps biexciton lifetime (τ_{xx}) measured in isolated PbS QDs (39) (Fig. 1B).

The structurally well-characterized interface of our electrolyte/PbS QD/single crystal TiO₂ system and the presence of a space charge field at the TiO₂ surface, which can quickly accelerate the injected electrons away from the interface, make this system particularly suitable to observe photocurrent collection from MEG in the adsorbed QDs. We synthesized (34) four QD samples with particle diameters of 9.9 ± 0.8 nm (standard deviation of transmission electron microscope data), 4.5 ± 0.3 nm, 3.1 ± 0.3 nm, and 2.5 ± 0.3 nm (fig. S1) with associated band gap energies (determined by the energy position in the absorbance spectra of the first exciton peak maxima) of 0.85 eV, 0.96 eV,

1.27 eV, and 1.39 eV, respectively. The semiconductor electrode is a nearly atomically flat anatase (001) surface that was imaged with AFM before and after exposure to MPA-capped PbS QDs. Figure 2A shows >100-nm terraces on the electrode surface before being uniformly coated by treatment with 4.5-nm MPA-capped PbS QDs [Fig. 2B; see also fig. S2 for QDs on rutile (001)]. The loose packing of the MPA-capped PbS QDs chemically linked to the TiO₂ surface suggests relatively smaller QD-QD interaction (or electronic coupling) when compared with a close-packed monolayer or multilayer films (26, 37).

Because the alignment of the QD excited states relative to the TiO₂ conduction band is size-dependent (Fig. 1A), we used photocurrent spectroscopy to resolve the sensitized photocurrents as a function of incident photon energy for each QD size. We measured the light power at each incident photon energy to calculate the incident photon-to-current efficiency (IPCE) spectra (Fig. 3) from the sensitized photocurrents according to Eq. 1 (where c is the speed of light and λ is wavelength).

$$\text{IPCE} = \frac{hc}{\lambda} \left[\frac{\text{photocurrent density } (\mu\text{A}/\text{cm}^2)}{\text{light power } (\mu\text{W}/\text{cm}^2)} \right] \quad (1)$$

The photocurrent response for QDs with E_g of 0.96 eV and larger (smaller diameter than 4.5 nm) showed distinct excitonic features at nearly the same photon energies observed in the absorbance spectra of the QDs suspended in water. Larger PbS QDs ($E_g = 0.85$ eV) did not sensitize the same anatase electrode at the energy of the first exciton because this excited state energy is more positive on the electrochemical scale than the TiO₂ conduction band energy (28, 40, 41). However, we observe sensitization from these QDs at 700 nm or 1.77 eV (Fig. 3A), indicating hot electron injection from higher QD excited states. A very recent study of PbSe QDs adsorbed on a rutile crystal in vacuum measured the time for hot electron injection to be extremely fast (50 fs) (37). Previous studies with similarly sized PbS QDs prepared by chemical bath deposition directly on

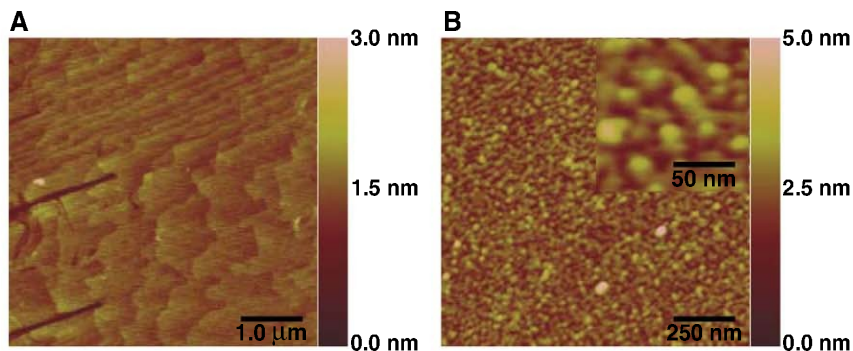


Fig. 2. AFM images of the anatase (001) electrode before and after the PbS QD adsorption procedure. **(A)** Bare electrode surface with an average terrace width of 350 nm with sporadic polishing damage indicated by ~2- to 5-nm grooves into the surface. **(B)** About 4.5-nm-diameter MPA-capped PbS QDs adsorbed on the same surface. (Inset) The loosely packed QDs at high resolution.

mesoporous TiO₂ films showed a photocurrent onset at about 800 nm or 1.5 eV (42), despite an onset of light absorption at ~1.2 eV. Thus, electron injection from the lowest excitonic states of PbS QDs into nanocrystalline TiO₂ was not observed previously in photocurrent spectra. This comparison suggests strong electronic coupling of PbS QD excited states to the TiO₂ conduction band in our system, resulting in facile charge injection and separation of the photoinjected electrons because of the space charge field present at the semiconductor-electrolyte interface. Therefore, multiple excitons produced from higher energy photons in the lowest energy PbS QD excited state should be collected as photocurrents with quantum yields greater than one.

For our planar electrodes covered with a single layer of QD sensitizers, the IPCE values are several orders of magnitude lower than those observed in typical sensitized mesoporous TiO₂ solar cells because of the low light absorption from the single layer of QDs. For this reason, the small

photocurrent response in the near-infrared (IR) spectral region was acquired by using lock-in detection and a chopped monochromatic light source. Although this technique is very sensitive for measuring small photocurrent signals, the quantification of MEG effects requires accurate measurement of the absorbed photon-to-current efficiency (APCE, Eq. 2). APCE values take into account the light-harvesting efficiency (LHE; Eq. 3), or light actually absorbed by the monolayer of QDs.

$$\text{APCE (\%)} = \text{IPCE (\%)} / \text{LHE (\%)} \quad (2)$$

$$\text{LHE (\%)} = 1 - 10^{-\text{Absorbance}} \quad (3)$$

Accurate determination of APCE values required accurate measurements of both the optical absorbance of the QDs adsorbed on the TiO₂ surface and the steady-state short circuit photocurrents at various incident photon energies (fig. S3, A to C). The absorbance measurements were performed on undoped semitransparent rutile (001)

TiO₂ single crystals with both sides polished (doped anatase and rutile crystals required for photocurrent measurements are not transparent) by using a dual-beam configuration in the ultraviolet-visible (UV-Vis) spectrometer (fig. S3D). Multiple regions of the rutile and anatase crystal surfaces, which were exposed to the same QD solutions at the same time, were imaged with AFM in order to assure that the densities of QDs on all the surfaces were nearly identical to those shown in Fig. 2B (also fig. S2). The absorbance spectra of the PbS QDs adsorbed on the rutile single crystal were similar to the solution absorbance spectra (fig. S4). Because of the larger band gap for the anatase polymorph compared with that of rutile (3.2 eV versus 3.0 eV), anatase was used for the MEC measurements to eliminate any contribution from direct excitation of TiO₂ to the sensitized photocurrent signal at the wavelengths where MEG would be expected.

Figure 4, A and B, shows the calculated APCE values both as a function of excitation energy and the ratio of the excitation energy to the nanocrystal band gap ($E_{\text{hv}}/E_{\text{g}}$) for the three sizes of PbS QDs. The APCE values, not adjusted for solution absorbance or reflections from the cell window and crystal surface, remained nearly constant for each QD sample at $70 \pm 13\%$ [standard deviation of photocurrent data (34)] from 1.6 eV up to an absolute photon energy of 2.5 eV (Fig. 4A). No increase in the quantum yields indicative of MEC was observed despite crossing the threshold of illumination with photon energies of twice the band gap for the 4.5-nm PbS QDs ($0.96 \times 2 = 1.92$ eV). However, at 2.8 and 3.1 eV illumination, the QDs with $E_{\text{g}} = 0.96$ eV (corresponding to photon energies of 2.9 and 3.2 times the band gap) exhibited APCE values that exceeded unity. There are also indications that APCE values, uncorrected for reflection and absorption losses, approach or exceed 100% at the highest photon energies for QDs with $E_{\text{g}} = 1.27$ and 1.39 eV; however, these values remain within the experimental error of the lower energy photocurrent measurements.

An additional sample of PbS nanocrystals with $E_{\text{g}} = 0.94$ eV was synthesized in order to ascertain any sample dependence (43) on the APCE yields (plotted in Fig. 4, A and B) and to more precisely map out the photon energy dependence of the MEC yields. The absolute magnitudes of the APCEs in this sample are smaller by 5 to 10% and 20 to 40% at photon energies in the non-MEC and MEC collection regions, respectively, but still nearly double at the higher (relative to lower) photon energies. We emphasize that in order to observe APCE values of over 100% the anatase electrode surface must not be contaminated and should exhibit large (>50 nm), nearly atomically flat terraces in AFM images. Therefore, in addition to any possible QD sample to sample variation in the MEC yields, we consider the condition of the electrode surface to be paramount in obtaining high and reproducible sensitized photocurrents.

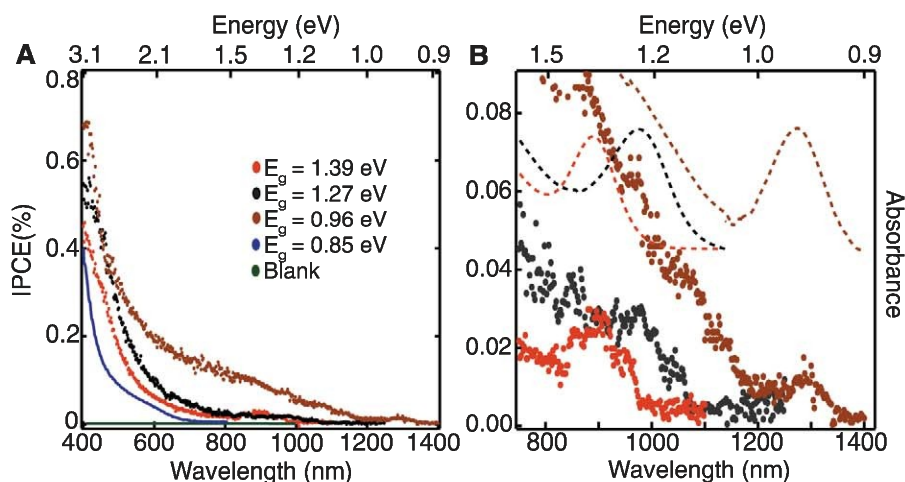


Fig. 3. IPCE spectra of various band gap PbS QDs adsorbed on an anatase (001) electrode. Photocurrents were acquired in an aqueous electrolyte (0.5 M Na₂S and 0.01 M S in 0.1 M NaOH) at short circuit in a two-electrode configuration versus a platinum wire. (A) IPCE spectra for each QD size. The green trace represents the bare anatase (001) photocurrent response. (B) IPCE spectra displaying the near-IR region to compare the photocurrent (solid dots) and QD absorbance in water (dashed lines).

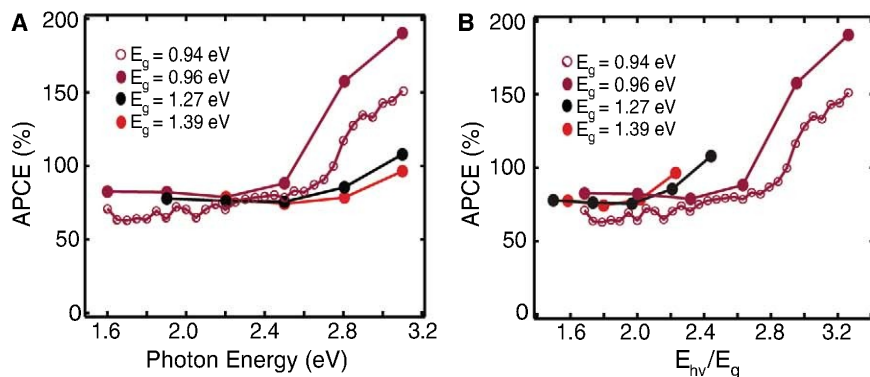


Fig. 4. APCE values as a function of the illumination energy. (A) APCE versus the absolute incident photon energy. (B) APCE versus the incident photon energy divided by the QD band gap energy (indicating the multiples of the band gap). Adjustments that would increase the APCE because of solution absorption and reflection from the cell window and TiO₂ crystal were not performed.

There are conflicting reports regarding the energy threshold for MEG and the extent to which quantum yields exceed unity in quantum-confined systems in solution measured with optical methods (43). However, there are substantial differences between optically and photoelectrochemically measured MEG. MEC in our photoelectrochemical photovoltaic system is calculated from very straightforward measurements of steady-state currents, photon fluxes, and optical absorption. Detection of MEG in isolated colloidal QDs using ultrafast optical techniques requires complex data analysis and may be complicated by artifacts associated with trap states, charging of the QDs, and multiple-photon absorption in a single QD that could result in higher apparent MEG yields (43). The photocurrents we measured exhibited no short time scale transients associated with charge trapping and detrapping of carriers (fig. S3, A to C). Furthermore, steady-state MEC photocurrents (APCE = 170%) were sustained for 8 hours of continuous illumination with $4.3 \mu\text{W}/\text{cm}^2$ of 3.1-eV incident photons; under these conditions, each QD undergoes nearly 1000 turnovers or on average about one multiple electron injection and collection every minute.

Although our >100% APCE values are higher and commence at slightly lower energy than the optically determined MEG yields for isolated colloidal PbS QDs (10), it was recently shown that the internal gain in PbS QD photoconductive photodetectors increased at $E_{\text{in}}/E_g = 2.7$ and nearly doubled at $E_{\text{in}}/E_g = 3.2$ (25). Despite the different mechanisms governing current flow in the two systems, the manifestation of MEG at similar values of E_{in}/E_g and the similar magnitudes of the yields are notable. The lower bound of the APCE values for our PbS QDs, demonstrating MEC at an absolute incident photon energy of 3.1 eV, is about 15 to 30% greater than MEG yields for oleic acid-capped PbS QDs ($E_g = 0.85$ eV) in tetrachloroethylene that was studied optically by Nozik and co-workers (10). The different dielectric environment of MPA-capped PbS QDs adsorbed on TiO_2 is one possible cause for the difference in absolute magnitude between our APCE values and MEG yields reported in typical solution spectroscopic measurements (i.e., water and a TiO_2 surface versus QDs capped with long-chain hydrocarbon surfactants in nonaqueous solutions). However, it appears that onset of MEC occurs at about $2.5 \pm 0.25 E_g$ for the QD sizes studied herein, in agreement with the onset of MEG determined optically with InP QDs with various band gaps (14). At present, optical measurements of MEG from PbS QDs capped with short-chain thiols in an aqueous medium are not available for direct comparison to our results.

The results presented herein are encouraging for the future design and development of photovoltaic devices that exploit MEG and MEC to surpass the Shockley-Queisser efficiency limit and approach the ideal single MEG absorber efficiency of 45% (7). However, it remains unclear to what extent MEC can improve the power con-

version efficiency in a thin film or QD-sensitized device, especially if the onset of MEC is at nearly three times the QD band gap.

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Supporting Online Material

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Materials and Methods
SOM Text
Figs. S1 to S4
Tables S1 to S4
References

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Allosteric Supramolecular Triple-Layer Catalysts

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Allosteric regulation of organometallic catalysts could allow for greater control over reactions. We report an allosteric supramolecular structure in which a monometallic catalytic site has been buried in the middle layer of a triple-layer complex. Small molecules and elemental anions can open and close this complex and reversibly expose and conceal the catalytic center. The ring-opening polymerization of ϵ -caprolactone can be turned on by the in situ opening of the triple-layer complex and then completely turned off by reforming it through the abstraction of Cl^- , the allosteric effector agent, without appreciable loss of catalytic activity. This process can regulate the molecular weights of the resulting polymers.

Supramolecular coordination complexes, in which multiple weak bonding interactions control structure, have found several applications, including catalysis (1–7), recognition of small molecules (8–11), and facilitated small-molecule transport (12). Such systems can encapsulate molecules (3), and the nanoscale environment created by a supramolecular complex can increase the speed of a chemical reaction (4, 5), alter reaction routes (6), control stereochemistry (5), affect oligomerization processes (7), or extend the lifetimes of highly reactive molecules such as

cyclobutadiene (13). An important goal is to regulate the activity of catalysts through the addition of small molecules that change the supramolecular structure of the catalyst and in turn control catalytic reaction rates and product distributions (14–17), much like the behavior of many allosteric enzymes. Herein, we present a proof-of-concept example of such regulation for a living polymerization catalyst and demonstrate control over polymer growth and molecular weight.

There are three general methods for synthesizing supramolecular coordination complexes (18),

termed the directional bonding (DBA) (19, 20), symmetry interaction (SIA) (21–23), and weak-link (WLA) approaches (24–26). The DBA and SIA yield structurally rigid complexes, whereas the WLA produces rigid intermediates, which can be chemically interconverted with structurally flexible cage-like products. We have used such interconversions through the WLA to synthesize and study a class of multimetallic allosteric complexes for molecular recognition and catalytic purposes (2, 24–26). Thus far, all allosteric supramolecular systems developed have relied on homoligated macrocycles or tweezers with pockets based upon catalysts that function in a bimetallic fashion. However, if the repertoire of allosteric systems could be extended from bimetallic to monometallic structures, myriad possibilities in the area of allosteric catalysis would result. Ideally, one would like access to a structure with a single catalytic site that is blocked above and below by chemically inert entities that can be opened and closed through chemical stimuli at remote regulatory sites.

We report the synthesis of a triple-layer complex (TLC), composed of two transition metal nodes, two chemically inert blocking exterior layers, and a single catalytically active interior Al(III)-salen complex, which can act as a ring-opening polymerization catalyst with ϵ -caprolactone (ϵ -CL) as the substrate (Fig. 1A). This complex was synthesized through the WLA and a halide-induced ligand rearrangement process (HILR)

(25, 27) and can be reversibly activated and deactivated through small-molecule or elemental anion effector reactions that assemble and disassemble the trilayer structures.

We initially studied the coordination chemistry of previously synthesized dirhodium complex **3** (Fig. 1B) (28). This complex can be reversibly interconverted with the triple-layer structure **4**, by the abstraction and subsequent addition of Cl^- . To realize the targeted triple-layer catalyst structure, we had to design a system that did not involve a Rh–O linkage as in compound **4**; this linkage is too easily broken by relatively weakly binding ligands (18, 24, 25) and presents a functional group incompatibility issue with many catalytic reactions. To synthesize a more robust structure, we replaced the ether moiety with an amine, which forms a stronger coordination bond with Rh(I) than the ether. The amine-containing, semi-open complex **7a**, a neutral species, was synthesized in 97% yield by reacting one equivalent (equiv) of $[\text{Rh}(\text{cod})\text{Cl}]_2$ (cod = 1,5-cyclooctadiene) with two equiv of PN ligand **6a** and one equiv of PS bidentate ligand **1** in dry CH_2Cl_2 at room temperature (Fig. 1B). All analytical data, including ^1H nuclear magnetic resonance (NMR) spectroscopy, $^{31}\text{P}\{^1\text{H}\}$ NMR spectroscopy, and ESI-MS (electrospray ionization mass spectrometry) are in full agreement with the proposed formulation [see supporting online material (SOM) for more details]. For example, the $^{31}\text{P}\{^1\text{H}\}$ NMR spectrum of **7a** exhibits highly diagnostic resonances at δ 72.95 (dd, $J_{\text{Rh-P}} = 184.68$ Hz, $J_{\text{P-P}} = 42.12$ Hz) and 27.31 (dd, $J_{\text{Rh-P}} = 166.86$ Hz, $J_{\text{P-P}} = 42.12$ Hz), indicative of the two inequivalent phosphorus atoms in the semi-open Rh(I) complex. A 2D $^{31}\text{P}\{^1\text{H}\}$ correlation spectroscopy NMR study also shows that the two resonances couple with each other (see SOM). The solid-state structure of **7a**, determined by a single-crystal x-ray diffraction study, is consistent with the proposed solution structure (Fig. 2, A and B). Each

of the Rh(I) centers is coordinated by κ^2 -PS, κ^1 -PN, and Cl^- moieties in a distorted square-planar geometry with P(2)–Rh(1)–Cl(1) and S(1)–Rh(1)–Cl(1) angles of $90.903(18)^\circ$ and $86.064(18)^\circ$, respectively.

Complex **7a** was transformed into the closed triple-layer structure **8a** through a reaction with NaBARf (BARf = tetrakis[(3,5-trifluoromethyl)phenyl]borate) or $\text{LiB}(\text{C}_6\text{F}_5)_4\cdot\text{Et}_2\text{O}$ halide abstracting reagents (29). The $^{31}\text{P}\{^1\text{H}\}$ NMR spectrum of closed triple-layer complex **8a** exhibits two resonances, which appear as doublets of doublets δ 66.73 (dd, $J_{\text{Rh-P}} = 178.20$ Hz, $J_{\text{P-P}} = 42.12$ Hz) and 49.95 (dd, $J_{\text{Rh-P}} = 173.34$ Hz, $J_{\text{P-P}} = 42.12$ Hz), respectively (Fig. 3A). Complex **8a** was also characterized in the solid-state by a single-crystal x-ray diffraction study, which is consistent with the proposed solution structure (Fig. 2, C and D). The two exterior phenyl and one interior phenyl moieties are almost cofacially aligned with respect to one another, suggesting that one could use this approach to create a complex with a chemically active pocket with blocking moieties positioned above and below the pocket. In the solid state, the closest distance between the interior phenyl group and the exterior blocking ligand was 3.3342 (56) Å. Two-dimensional (2D) and selective 1D ^1H NOESY (nuclear Overhauser effect spectroscopy) experiments also confirmed that complex **8a** maintains its triple-layer structure in solution (Fig. 3, B and C).

Based on experiments with model ligands **1** and **6a**, more sophisticated ligands **5** and **6b** (Fig. 1B) were designed and used to synthesize the corresponding neutral semi-open complex **7b**. Ligands **5** and **6b** were prepared from commercially available compounds, 5-bromo-2-hydroxybenzaldehyde and *N,N*-bis(4-bromophenyl)-amine, respectively (see SOM). At room temperature, a THF solution of $[\text{Rh}(\text{coe})_2\text{Cl}]_2$ (44.0 μmol , coe = cyclooctene) was added to a THF solution of one equiv of compound **5** (44.0 μmol) and two equiv of com-

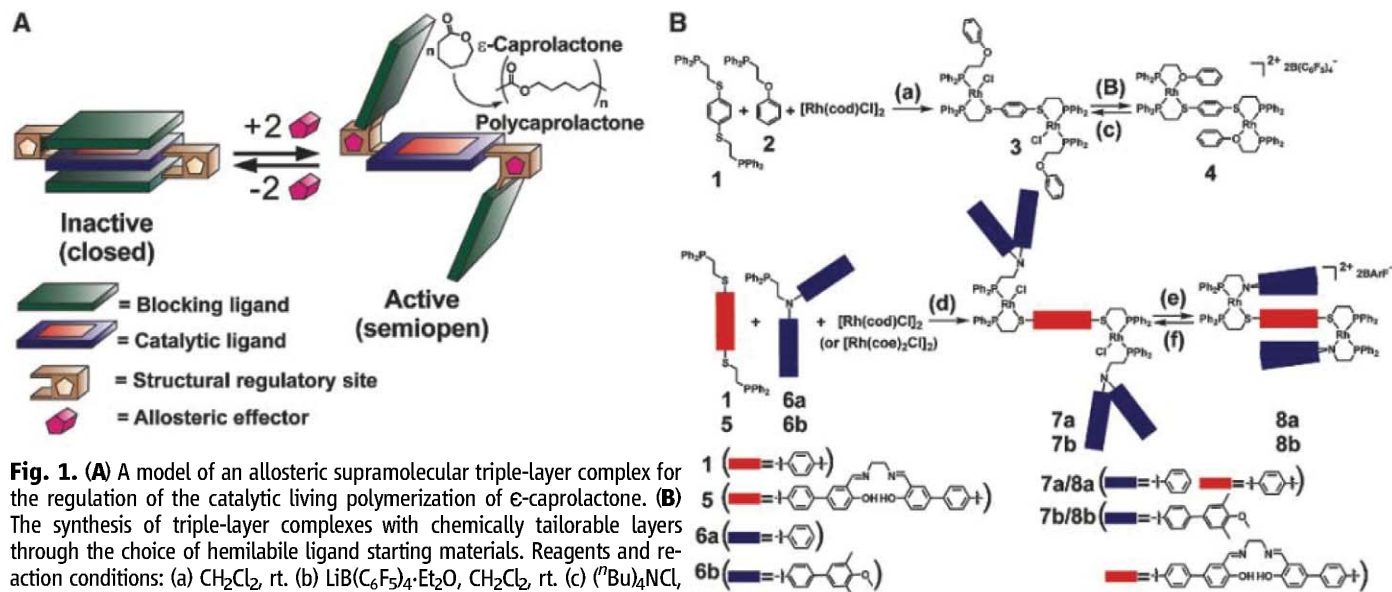


Fig. 1. (A) A model of an allosteric supramolecular triple-layer complex for the regulation of the catalytic living polymerization of ϵ -caprolactone. **(B)** The synthesis of triple-layer complexes with chemically tailorable layers through the choice of hemilabile ligand starting materials. Reagents and reaction conditions: (a) CH_2Cl_2 , rt. (b) $\text{LiB}(\text{C}_6\text{F}_5)_4\cdot\text{Et}_2\text{O}$, CH_2Cl_2 , rt. (c) $(t\text{Bu})_4\text{NCl}$, CH_2Cl_2 , rt. (d) THF, rt. (e) NaBARf, CH_2Cl_2 , rt. (f) $(t\text{Bu})_4\text{NCl}$, CH_2Cl_2 , rt.

pound **6b** (88.0 μmol) under a N_2 atmosphere. The product was concentrated under vacuum and recrystallized with THF and hexanes to yield neutral semi-open **7b** in 92% yield (Fig. 1B). Complex **7b** could be readily transformed into the closed triple-layer structure by introducing it to NaBARf or $\text{LiB}(\text{C}_6\text{F}_5)_4\text{-Et}_2\text{O}$. The molecular structure of complex **8b** was confirmed by ESI-MS and $^{31}\text{P}\{^1\text{H}\}$ NMR spectroscopy. A parent peak in the mass spectrum of **8b** was observed at 1206.3536 m/z ($[\text{M}-2\text{BARf}]^{2+}$), and the $^{31}\text{P}\{^1\text{H}\}$ NMR spectrum of **8b** exhibited resonances at δ 67.09 (dd, $J_{\text{Rh-P}} = 178.18$ Hz, $J_{\text{P-P}} = 42.11$ Hz) and 50.29 (dd, $J_{\text{Rh-P}} = 173.32$ Hz, $J_{\text{P-P}} = 42.11$ Hz), which were assigned to the two magnetically and chemically distinct P atoms.

The catalytically active triple-layer structure **9** (Fig. 4A) was obtained in 89% yield via reaction of compound **7b** with AlEt_3 in dry toluene followed by addition of ethanol (EtOH). The $^{31}\text{P}\{^1\text{H}\}$ NMR spectrum of **9** exhibited resonances at δ 73.16 (dd, $J_{\text{Rh-P}} = 186.28$ Hz, $J_{\text{P-P}} = 42.11$ Hz) and 27.69 (dd, $J_{\text{Rh-P}} = 166.84$ Hz, $J_{\text{P-P}} = 40.50$ Hz), indicating that the compound has a semi-open structure. In addition, ^1H NMR spectroscopy and ESI-MS of compound **9** confirmed that it bears a $\text{CH}_3\text{CH}_2\text{O-Al}$ group. Reaction of two equiv of NaBARf or $\text{LiB}(\text{C}_6\text{F}_5)_4\text{-Et}_2\text{O}$ with compound **9** in CH_2Cl_2 afforded a fully closed triple-layer structure **10** in quantitative yield. Resonances at δ 66.86 (dd, $J_{\text{Rh-P}} = 179.80$ Hz, $J_{\text{P-P}} = 42.11$ Hz) and 50.07 (dd, $J_{\text{Rh-P}} = 171.70$ Hz, $J_{\text{P-P}} = 42.11$ Hz) were observed in the $^{31}\text{P}\{^1\text{H}\}$ NMR spectrum of **10**, which indicate a fully closed structure. A selective 1D NOESY NMR spectroscopic study of **10** is consistent with the characterization of the complex as a triple-layer structure. Specifically, a through-space interaction occurs between the terminal methyl group of the ethoxy moiety and the methyl groups of the blocking layers (see SOM). When two equiv of Cl^- (or CD_3CN) were added to a CH_2Cl_2 solution of compound **10** at room temperature, the corresponding semi-open complex **9** immediately and quantitatively formed without destroying the $\text{Al(III)-OCH}_2\text{CH}_3$ -salen moiety, as evidenced by ESI-MS and ^1H and $^{31}\text{P}\{^1\text{H}\}$ NMR spectroscopy in CD_2Cl_2 .

The catalytic properties of closed compound **10** and semi-open compound **9** were evaluated in terms of a ring-opening polymerization of ϵ -CL (Fig. 4B, C). In a typical experiment, catalyst **9** (2.09 mM) was added to a toluene- d_8 solution of ϵ -CL (331 or 541 mM), and the formation of the product, polycaprolactone (PCL), was monitored by ^1H NMR spectroscopy as a function of time (Fig. 4B) (30). The semi-open compound **9** is active and can quantitatively polymerize all of the ϵ -CL monomer. The closed compound **10** is initially almost completely inactive under identical conditions, but after about 100 hours of catalysis, residual activity is observed (about 7% of **9**), which is caused by decomposition of **10** (confirmed by $^{31}\text{P}\{^1\text{H}\}$ NMR spectroscopy). The polymers produced by the reactions were characterized by ^1H , $^{13}\text{C}\{^1\text{H}\}$ NMR spectroscopy, gel permeation

chromatography (GPC), and differential scanning calorimetry (DSC) to confirm the molecular structure of the polymer and to determine their number average molecular weights (M_n), weight average molecular weights (M_w), and polydispersity indices (PDI). Every polymer produced by the TLC showed a relatively narrow molecular weight distribution (PDI: 1.1 $\sim$ 1.2) and plausible M_n s

and M_w s, compared with theoretical ones (table S1). For example, in the case of 541 mM ϵ -CL at 90°C, the product had a PDI of 1.18 and a M_w of 35,400. Polymers with $M_w = 20,800$ and 35,400 exhibited T_m s of 47.8 and 50.3°C, respectively (31).

We could allosterically regulate the catalytic activity of the supramolecular catalyst with small

Fig. 2. Crystal structures of the compounds **7a** (A and B) and **8a** (C and D), drawn with 50% thermal ellipsoid probability: (A) and (C), side view; (B) and (D), top view. Hydrogen atoms, solvent molecules, and counter ions have been omitted for clarity. Labeling and coloring schemes are as follows: Rh, orange; P, green; S, yellow; N, dark brown; Cl, blue; C, gray.

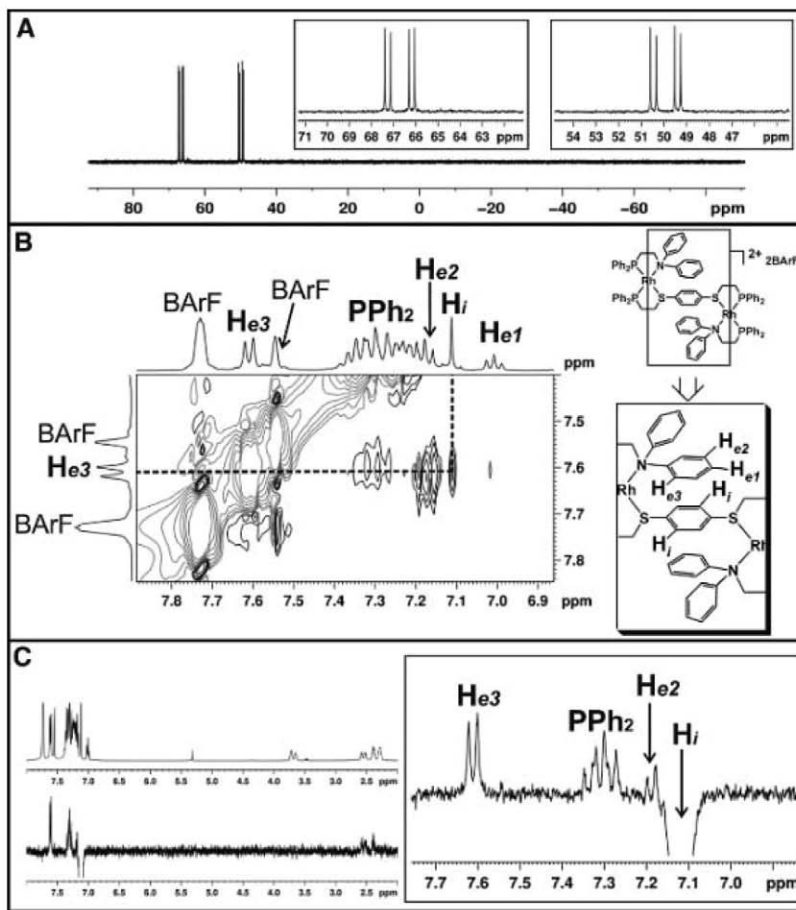
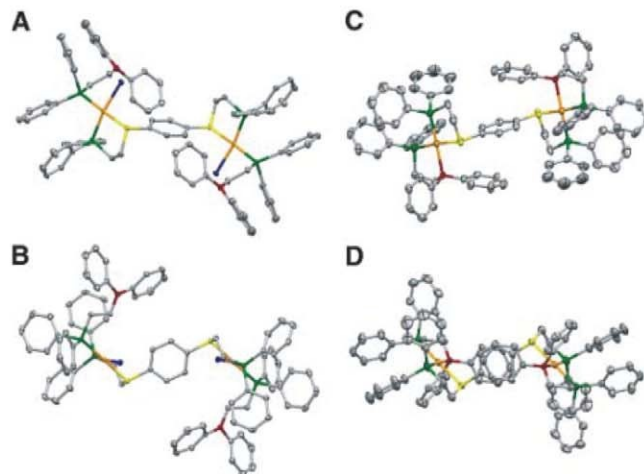


Fig. 3. (A) $^{31}\text{P}\{^1\text{H}\}$ NMR spectrum of compound **8a**. The inset shows that the resonances are doublets of doublets. (B) An aromatic region of a 2D NOESY NMR spectrum of **8a**. The spectrum shows the nuclear Overhauser effect (NOE) between interior (H_i) and exterior (H_{e3}) protons. (C) ^1H NMR spectrum of **8a** and the corresponding selective 1D NOESY NMR trace which was obtained through irradiation at the frequency of H_{e3} . (Inset) Aromatic region of the 1D NOESY spectrum.

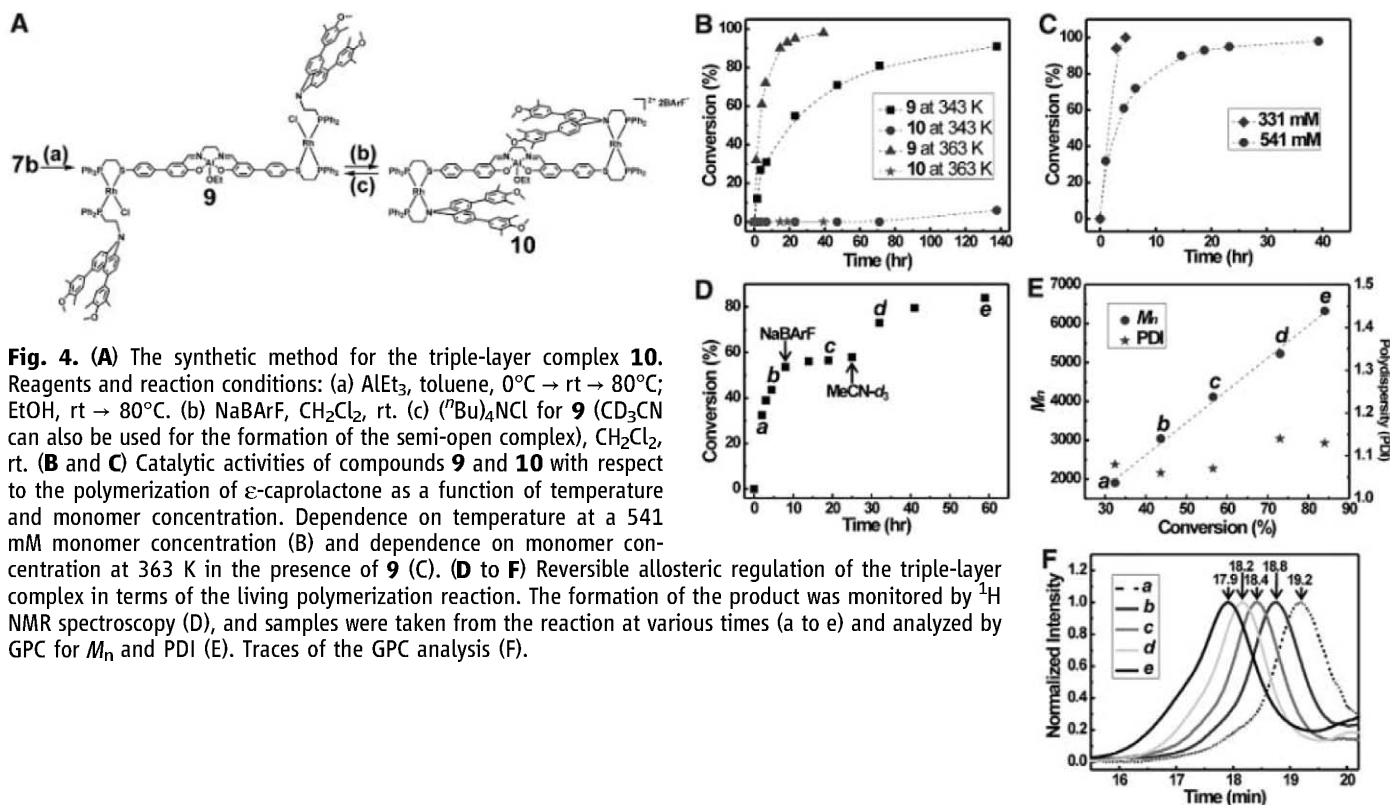


Fig. 4. (A) The synthetic method for the triple-layer complex **10**. Reagents and reaction conditions: (a) AlEt_3 , toluene, $0^\circ\text{C} \rightarrow \text{rt} \rightarrow 80^\circ\text{C}$; EtOH , $\text{rt} \rightarrow 80^\circ\text{C}$. (b) NaBARf , CH_2Cl_2 , rt . (c) $(^t\text{Bu})_4\text{NCl}$ for **9** (CD_3CN can also be used for the formation of the semi-open complex), CH_2Cl_2 , rt . (B and C) Catalytic activities of compounds **9** and **10** with respect to the polymerization of ϵ -caprolactone as a function of temperature and monomer concentration. Dependence on temperature at a 541 mM monomer concentration (B) and dependence on monomer concentration at 363 K in the presence of **9** (C). (D to F) Reversible allosteric regulation of the triple-layer complex in terms of the living polymerization reaction. The formation of the product was monitored by ^1H NMR spectroscopy (D), and samples were taken from the reaction at various times (a to e) and analyzed by GPC for M_n and PDI (E). Traces of the GPC analysis (F).

molecules (Fig. 4D). As a proof-of-concept experiment, the catalyst was deactivated by adding a stoichiometric amount of the Cl^- abstracting agent, NaBARf , which resulted in the clean formation of the fully closed complex **10**, as evidenced by $^3\text{P}\{^1\text{H}\}$ NMR spectroscopy. Addition of acetonitrile to **10** yields the semi-open structure again and reactivates the catalyst, allowing ϵ -CL to react with the Al(III) -salen moiety resulting in polymer growth (Fig. 4D). To characterize this process, a 2-mL sample was taken at various times from the reaction solution and characterized by ^1H NMR spectroscopy and GPC (Fig. 4 E and F). A linear relation between percentage of substrate conversion to product and M_n of the polymer was observed, which confirms that the catalyst maintains its catalytic activity during the allosteric regulation process. Reactivation of the catalyst results in increased M_w , because of the living nature of the polymerization and not just the generation of additional polymer of a M_w comparable to the first stage of activation.

Allosteric modulation of enzymatic catalysis kinetics is used for many purposes, including the control of RNA polymerase activity during transcription (32), signal transduction in cellular structures (33), and energy regulation (34). The allosteric triple-layer catalyst reported herein is a step toward a new class of supramolecular systems that should allow one to regulate a wide variety of chemical reactions with activities, regioselectivities, and perhaps stereoselectivities that can be controlled with small molecule and elemental anion effectors. Indeed, the ability to introduce a variety of functional groups into an A-B-A type of triple-layered structure via coordination chemistry and the WLA

is a powerful and potentially general way of creating sterically protected catalytically active centers that can be completely activated and deactivated in situ without substantial loss of catalytic activity.

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Isoprenoid Pathway Optimization for Taxol Precursor Overproduction in *Escherichia coli*

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Taxol (paclitaxel) is a potent anticancer drug first isolated from the *Taxus brevifolia* Pacific yew tree. Currently, cost-efficient production of Taxol and its analogs remains limited. Here, we report a multivariate-modular approach to metabolic-pathway engineering that succeeded in increasing titers of taxadiene—the first committed Taxol intermediate—approximately 1 gram per liter (~15,000-fold) in an engineered *Escherichia coli* strain. Our approach partitioned the taxadiene metabolic pathway into two modules: a native upstream methylerythritol-phosphate (MEP) pathway forming isopentenyl pyrophosphate and a heterologous downstream terpenoid-forming pathway. Systematic multivariate search identified conditions that optimally balance the two pathway modules so as to maximize the taxadiene production with minimal accumulation of indole, which is an inhibitory compound found here. We also engineered the next step in Taxol biosynthesis, a P450-mediated 5 α -oxidation of taxadiene to taxadien-5 α -ol. More broadly, the modular pathway engineering approach helped to unlock the potential of the MEP pathway for the engineered production of terpenoid natural products.

Taxol (paclitaxel) and its structural analogs are among the most potent and commercially successful anticancer drugs (1). Taxol was first isolated from the bark of the Pacific yew tree (2), and early-stage production methods required sacrificing two to four fully grown trees to secure sufficient dosage for one patient (3). Taxol's structural complexity limited its chemical synthesis to elaborate routes that required 35 to

51 steps, with a highest yield of 0.4% (4–6). A semisynthetic route was later devised in which the biosynthetic intermediate baccatin III, isolated from plant sources, was chemically converted to Taxol (7). Although this approach and subsequent plant cell culture-based production efforts have decreased the need for harvesting the yew tree, production still depends on plant-based processes (8), with accompanying limitations on productivity and scalability. These methods of production also constrain the number of Taxol derivatives that can be synthesized in the search for more efficacious drugs (9, 10).

Recent developments in metabolic engineering and synthetic biology offer new possibilities for the overproduction of complex natural products by optimizing more technically amenable micro-

bial hosts (11, 12). The metabolic pathway for Taxol consists of an upstream isoprenoid pathway that is native to *Escherichia coli* and a heterologous downstream terpenoid pathway (fig. S1). The upstream methylerythritol-phosphate (MEP) or heterologous mevalonic acid (MVA) pathways can produce the two common building blocks, isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP), from which Taxol and other isoprenoid compounds are formed (12). Recent studies have highlighted the engineering of the above upstream pathways to support the biosynthesis of heterologous isoprenoids such as lycopene (13, 14), artemisinic acid (15, 16), and abietadiene (17, 18). The downstream taxadiene pathway has been reconstructed in *E. coli* and *Saccharomyces cerevisiae* together with the overexpression of upstream pathway enzymes, but to date titers have been limited to less than 10 mg/liter (19, 20).

The above rational metabolic engineering approaches examined separately either the upstream or the downstream terpenoid pathway, implicitly assuming that modifications are additive (a linear behavior) (13, 17, 21). Although this approach can yield moderate increases in flux, it generally ignores nonspecific effects, such as toxicity of intermediate metabolites, adverse cellular effects of the vectors used for expression, and hidden pathways and metabolites that may compete with the main pathway and inhibit the production of the desired molecule. Combinatorial approaches can overcome such problems because they offer the opportunity to broadly sample the parameter space and bypass these complex nonlinear interactions (21–23). However, combinatorial approaches require high-throughput screens, which are often not available for many desirable natural products (24).

Considering the lack of a high-throughput screen for taxadiene (or other Taxol pathway intermediate), we resorted to a focused combi-

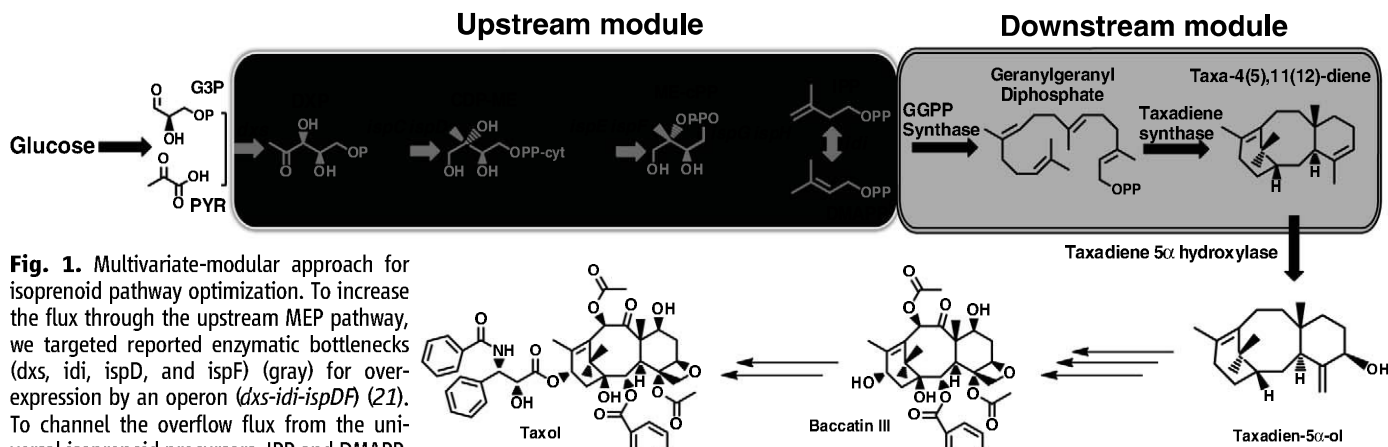


Fig. 1. Multivariate-modular approach for isoprenoid pathway optimization. To increase the flux through the upstream MEP pathway, we targeted reported enzymatic bottlenecks (dxs, idi, ispD, and ispF) (gray) for overexpression by an operon (*dxs-idi-ispDF*) (21). To channel the overflow flux from the universal isoprenoid precursors, IPP and DMAPP, toward Taxol biosynthesis, we constructed a synthetic operon of downstream genes GGPP synthase (G) and taxadiene synthase (T) (37). Both pathways were placed under the control of inducible promoters in order to control their relative gene expression. In the *E. coli* metabolic network, the MEP isoprenoid pathway is initiated by the condensation of the precursors glyceraldehyde-3 phosphate (G3P) and pyruvate

(PYR) from glycolysis. The Taxol pathway bifurcation starts from the universal isoprenoid precursors IPP and DMAPP to form geranylgeranyl diphosphate, and then the taxadiene. The cyclic olefin taxadiene undergoes multiple rounds of stereospecific oxidations, acylations, and benzylation to form the late intermediate Baccatin III and side chain assembly to, ultimately, form Taxol.

natorial approach, which we term “multivariate-modular pathway engineering.” In this approach, the overall pathway is partitioned into smaller modules, and the modules’ expression are varied simultaneously—a multivariate search. This approach can identify an optimally balanced pathway while searching a small combinatorial space. Specifically, we partition the taxadiene-forming pathway into two modules separated at IPP, which is the key intermediate in terpenoid biosynthesis. The first module comprises an eight-gene, upstream, native (MEP) pathway of which the expression of only four genes deemed to be rate-limiting was modulated, and the second module comprises a two-gene, downstream, heterologous pathway to taxadiene (Fig. 1). This modular approach allowed us to efficiently sample the main parameters affecting pathway flux without the need for a high-throughput screen and to unveil the role of the metabolite indole as inhibitor of isoprenoid pathway activity. Additionally, the multivariate search revealed a highly nonlinear taxadiene flux landscape with a global maximum exhibiting a 15,000-fold increase in taxadiene production over the control, yielding 1.02 ± 0.08 g/liter (SD) taxadiene in fed-batch bioreactor fermentations.

We have further engineered the P450-based oxidation chemistry in Taxol biosynthesis in *E. coli* to convert taxadiene to taxadien-5 α -ol and provide the basis for the synthesis of subsequent metabolites in the pathway by means of similar cytochrome P450 (CYP450) oxidation chemistry. Our engineered strain improved taxadiene-5 α -ol production by 2400-fold over the state of the art with yeast (25). These advances unlock the potential of microbial processes for the large-scale production of Taxol or its derivatives and thousands of other valuable terpenoids.

The multivariate-modular approach in which various promoters and gene copy-numbers are combined to modulate diverse expression levels of upstream and downstream pathways of taxadiene synthesis is schematically described in fig. S2. A total of 16 strains were constructed in order to widen the bottleneck of the MEP pathway as well as optimally balance it with the downstream taxadiene pathway (26). The dependence of taxadiene accumulation on the upstream pathway for constant values of the downstream pathway is shown in Fig. 2A, and the dependence on the downstream pathway for constant upstream pathway strength is shown in Fig. 2B (table S1, calculation of the upstream and downstream pathway strength from gene copy number and promoter strength). As the upstream pathway expression increases in Fig. 2A from very low levels, taxadiene production also rises initially because of increased supply of precursors to the overall pathway. However, after an intermediate value further upstream pathway increases cannot be accommodated by the capacity of the downstream pathway. For constant upstream pathway expression (Fig. 2B), a maximum in downstream expression

was similarly observed owing to the rising edge to initial limiting of taxadiene production by low expression levels of the downstream pathway. At high (after peak) levels of downstream pathway expression, we were probably observing the negative effect on cell physiology of the high copy number.

These results demonstrate that dramatic changes in taxadiene accumulation can be obtained from

changes within a narrow window of expression levels for the upstream and downstream pathways. For example, a strain containing an additional copy of the upstream pathway on its chromosome under Trc promoter control (strain 8) (Fig. 2A) produced 2000-fold more taxadiene than one expressing only the native MEP pathway (strain 1) (Fig. 2A). Furthermore, changing the order of the genes in the downstream syn-

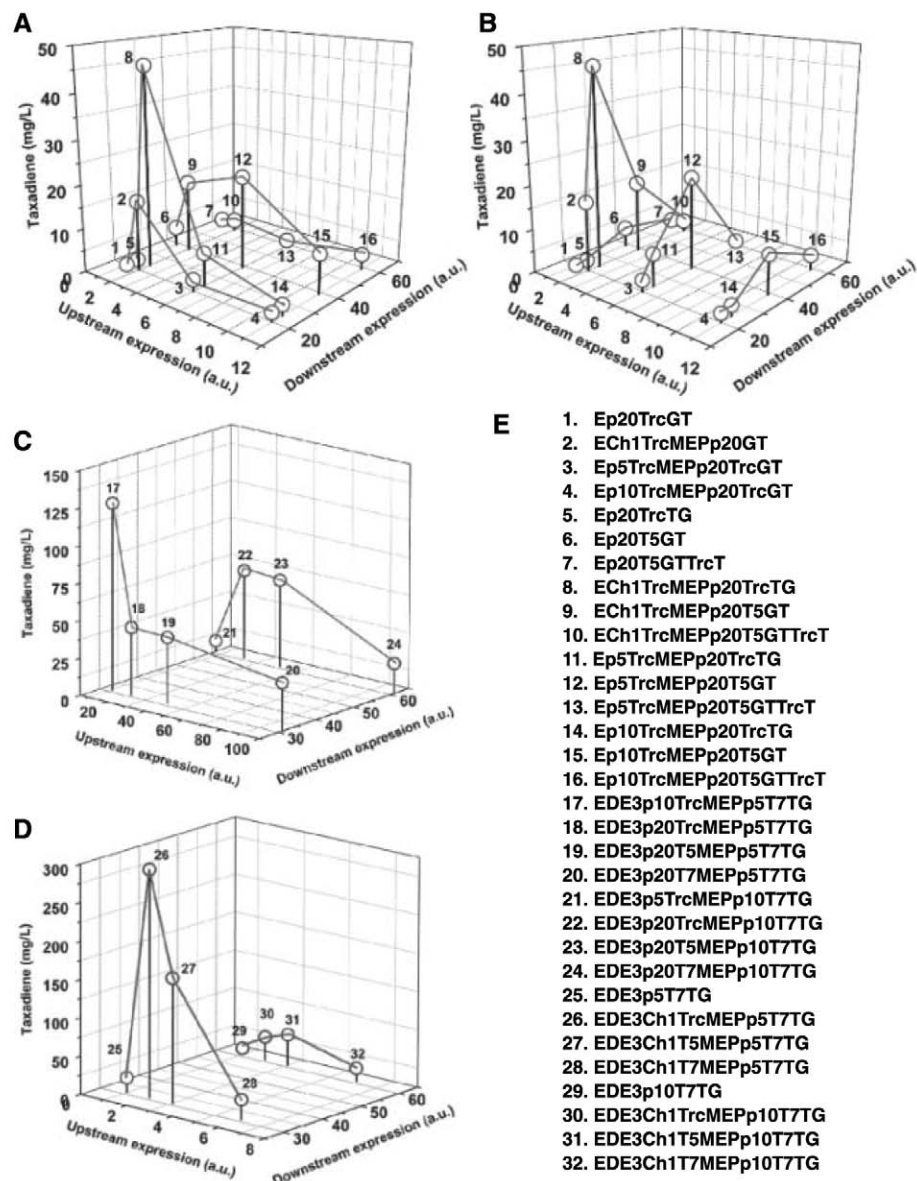


Fig. 2. Optimization of taxadiene production through regulating the expression of the upstream and downstream modular pathways. (A) Response in taxadiene accumulation to changes in upstream pathway strengths for constant values of the downstream pathway. (B) Dependence of taxadiene on the downstream pathway for constant levels of upstream pathway strength. (C) Taxadiene response from strains (17 to 24) engineered with high upstream pathway overexpressions (6 to 100 a.u.) at two different downstream expressions (31 a.u. and 61 a.u.). (D) Modulation of a chromosomally integrated upstream pathway by using increasing promoter strength at two different downstream expressions (31 a.u. and 61 a.u.). (E) Genotypes of the 32 strain constructs whose taxadiene phenotype is shown in Fig. 2, A to D. E, *E. coli* K12MG1655 Δ recA Δ enaA; EDE3, *E. coli* K12MG1655 Δ recA Δ enaA with DE3 T7 RNA polymerase gene in the chromosome; MEP, *dxs-idi-ispDF* operon; GT, GPP5-T5 operon; TG, T5-GPP5 operon; Ch1, 1 copy in chromosome; Trc, Trc promoter; T5, T5 promoter; T7, T7 promoter; p5, pSC101 plasmid; p10, p15A plasmid; and p20, pBR322 plasmid.

thetic operon from GT (GGPS-TS) to TG (TS-GGPS) resulted in a two- to threefold increase (strains **1** to **4** as compared with strains **5**, **8**, **11**, and **14**). Altogether, the engineered strains established that the MEP pathway flux can be substantial if an appropriate range of expression levels for the endogenous upstream and synthetic downstream pathway are searched simultaneously.

To provide ample downstream pathway strength while minimizing the plasmid-born metabolic burden (27), two new sets of four strains each were engineered (strains **17** to **20** and **21** to **24**), in which the downstream pathway was placed under the control of a strong promoter (T7) while keeping a relatively low number of five and 10 plasmid copies, respectively. The taxadiene maximum was maintained at high downstream strength (strains **21** to **24**), whereas a monotonic response was obtained at the low downstream pathway strength (strains **17** to **20**) (Fig. 2C). This observation prompted the construction of two additional sets of four strains each that maintained the same level of downstream pathway strength as before but expressed very low levels of the upstream pathway (strains **25** to **28** and **29** to **32**) (Fig. 2D). Additionally, the operon of the upstream pathway of the latter strain set was chromosomally integrated (fig S3). Not only was the taxadiene maximum recovered in these strains, albeit at very low upstream pathway levels, but a much greater taxadiene maximum was attained (~300 mg/liter). We believe that this significant increase can be attributed to a decrease in the cell's metabolic burden.

We next quantified the mRNA levels of 1-deoxy-D-xylulose-5-phosphate synthase (*dxs*) and taxadiene synthase (*TS*) (representing the upstream and downstream pathways, respectively) for the high-taxadiene-producing strains (**25** to **32** and **17** and **22**) that exhibited varying upstream and downstream pathway strengths (fig. S4, A and B) to verify our predicted expression strengths were consistent with the actual pathway levels. We found that *dxs* expression level correlates well with the upstream pathway strength. Similar correlations were found for the other genes of the upstream pathway: *idi*, *ispD*, and *ispF* (fig. S4, C and D). In downstream *TS* gene expression, an approximately twofold improvement was quantified as the downstream pathway strength increased from **31** to **61** arbitrary units (a.u.) (fig. S4B).

Metabolomic analysis of the previous strains led to the identification of a distinct metabolite by-product that inversely correlated with taxadiene accumulation (figs. S5 and S6). The corresponding peak in the gas chromatography-mass spectrometry (GC-MS) chromatogram was identified as indole through GC-MS, ^1H , and ^{13}C nuclear magnetic resonance (NMR) spectroscopy studies (fig. S7). We found that taxadiene synthesis by strain **26** is severely inhibited by exogenous indole at indole levels higher than ~100 mg/liter (fig. S5B). Further increasing the indole concentration also inhibited cell growth, with the level of

inhibition being very strain-dependent (fig. S5C). Although the biochemical mechanism of indole interaction with the isoprenoid pathway is presently unclear, the results in fig. S5 suggest a possible synergistic effect between indole and terpenoid compounds of the isoprenoid pathway in inhibiting cell growth. Without knowing the specific mechanism, it appears that strain **26** has mitigated the indole's effect, which we carried forward for further study.

In order to explore the taxadiene-producing potential under controlled conditions for the engineered strains, fed-batch cultivations of the three highest taxadiene accumulating strains (~60 mg/liter from strain **22**; ~125 mg/liter from strain **17**; and ~300 mg/liter from strain **26**) were carried out in 1-liter bioreactors (Fig. 3). The fed-batch cultivation studies were carried out as liquid-liquid two-phase fermentation using a 20% (v/v) dodecane overlay. The organic solvent was introduced to prevent air stripping of secreted taxadiene from the fermentation medium, as indicated by preliminary findings (fig. S8). In defined media with controlled glycerol feeding, taxadiene productivity increased to 174 ± 5 mg/liter (SD), 210 ± 7 mg/liter (SD), and 1020 ± 80 mg/liter (SD) for strains **22**, **17**, and **26**, respectively (Fig. 3A). Additionally, taxadiene production significantly affected the growth phenotype, acetate accumulation, and glycerol consumption [Fig. 3, B and D, and supporting online material (SOM) text]. Clearly, the high productivity and more robust

growth of strain **26** allowed very high taxadiene accumulation. Further improvements should be possible through optimizing conditions in the bioreactor, balancing nutrients in the growth medium and optimizing carbon delivery.

Having succeeded in engineering the biosynthesis of the "cyclase phase" of Taxol for high taxadiene production, we turned next to engineering the oxidation-chemistry of Taxol biosynthesis. In this phase, hydroxyl groups are incorporated by oxygenation at seven positions on the taxane core structure, mediated by CYP450-dependent monooxygenases (28). The first oxygenation is the hydroxylation of the C5 position, followed by seven similar reactions en route to Taxol (fig. S1) (29). Thus, a key step toward engineering Taxol-producing microbes is the development of CYP450-based oxidation chemistry in vivo. The first oxygenation step is catalyzed by a CYP450, taxadiene 5 α -hydroxylase, which is an unusual monooxygenase that catalyzes the hydroxylation reaction along with double-bond migration in the diterpene precursor taxadiene (Fig. 1).

In general, functional expression of plant CYP450 in *E. coli* is challenging (30) because of the inherent limitations of bacterial platforms, such as the absence of electron transfer machinery and CYP450-reductases (CPRs) and translational incompatibility of the membrane signal modules of CYP450 enzymes because of the lack of an endoplasmic reticulum. Recently, through transmembrane (TM) engineering and the gener-

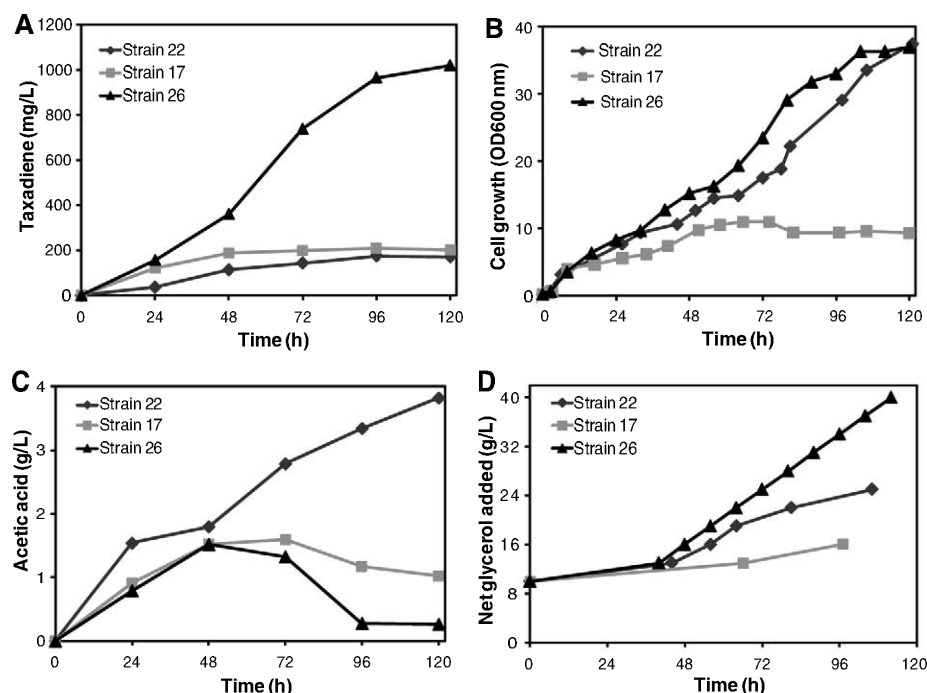


Fig. 3. Fed-batch cultivation of engineered strains in a 1-liter bioreactor. Time courses of (A) taxadiene accumulation, (B) cell growth, (C) acetic acid accumulation, and (D) total substrate (glycerol) addition for strains **22**, **17**, and **26** during 5 days of fed-batch bioreactor cultivation in 1-liter bioreactor vessels under controlled pH and oxygen conditions with minimal media and 0.5% yeast extract. After glycerol depletes to ~0.5 to 1 g/liter in the fermentor, 3 g/liter of glycerol was introduced into the bioreactor during the fermentation. Data are mean of two replicate bioreactors.

ation of chimera enzymes of CYP450 and CPR, some plant CYP450s have been expressed in *E. coli* for the biosynthesis of functional molecules (15, 31). Still, every plant CYP450 has distinct TM signal sequences and electron transfer characteristics from its reductase counterpart (32). Our initial studies were focused on optimizing the expression of codon-optimized synthetic taxadiene 5 α -hydroxylase by N-terminal TM engineering and generating chimera enzymes through translational fusion with the CPR redox partner from the *Taxus* species, *Taxus* CYP450 reductase (TCPR) (Fig. 4A) (29, 31, 33). One of the chimera enzymes generated, At24T5 α OH-tTCPR, was highly efficient in carrying out the first oxidation step, resulting in more than 98% taxadiene conversion to taxadien-5 α -ol and the byproduct 5(12)-Oxa-3(11)-cyclotaxane (OCT) (fig. S9A).

Compared with the other chimeric CYP450s, At24T5 α OH-tTCPR yielded twofold higher (21 mg/liter) production of taxadien-5 α -ol (Fig. 4B). Because of the functional plasticity of taxadiene 5 α -hydroxylase with its chimeric CYP450's enzymes (At8T5 α OH-tTCPR, At24T5 α OH-tTCPR, and At42T5 α OH-tTCPR), the reaction also yields a complex structural rearrangement of taxadiene into the cyclic ether OCT (fig. S9) (34). The by-product accumulated in approximately equal amounts (~24 mg/liter from At24T5 α OH-tTCPR) to the desired product taxadien-5 α -ol.

The productivity of strain 26-At24T5 α OH-tTCPR was significantly reduced relative to that of taxadiene production by the parent strain 26 (~300 mg/liter), with a concomitant increase in indole accumulation. No taxadiene accumulation was observed. Apparently, the introduction of an additional medium copy plasmid (10-copy, p10T7) bearing the At24T5 α OH-tTCPR construct disturbed the carefully engineered balance in the upstream and downstream pathway of strain 26 (fig. S10). Small-scale fermentations were carried out in bioreactors so as to quantify the alcohol production by strain 26-At24T5 α OH-tTCPR. The time course profile of taxadien-5 α -ol accumulation (Fig. 4C) indicates alcohol production of up to 58 ± 3 mg/liter (SD) with an equal amount of the OCT by-product produced. The observed alcohol production was approximately 2400-fold higher than previous production in *S. cerevisiae* (25).

The MEP pathway is energetically balanced and thus overall more efficient in converting either glucose or glycerol to isoprenoids (fig. S11). Yet, during the past 10 years many attempts at engineering the MEP pathway in *E. coli* in order to increase the supply of the key precursors IPP and DMAPP for carotenoid (21, 35), sesquiterpenoid (16), and diterpenoid (17) overproduction met with limited success. This inefficiency was attributed to unknown regulatory effects associated specifically with the expression of the MEP path-

way in *E. coli* (16). Here, we provide evidence that such limitations are correlated with the accumulation of the metabolite indole, owing to the non-optimal expression of the pathway, which inhibits the isoprenoid pathway activity. Taxadiene overproduction (under conditions of indole-formation suppression), establishes the MEP pathway as a very efficient route for biosynthesis of pharmaceutical and chemical products of the isoprenoid family (fig. S11). One simply needs to carefully balance the modular pathways, as suggested by our multivariate-modular pathway-engineering approach.

For successful microbial production of Taxol, demonstration of the chemical decoration of the taxadiene core by means of CYP450-based oxidation chemistry is essential (28). Previous efforts to reconstitute partial Taxol pathways in yeast found CYP450 activity limiting (25), making the At24T5 α OH-tTCPR activity levels an important step to debottleneck the late Taxol pathway. Additionally, the strategies used to create At24T5 α OH-tTCPR are probably applicable for the remaining monooxygenases that will require expression in *E. coli*. CYP450 monooxygenases constitute about one half of the 19 distinct enzymatic steps in the Taxol biosynthetic pathway. These genes show unusually high sequence similarity with each other (>70%) but low similarity (<30%) with other plant CYP450s (36), implying that these monooxygenases are amenable to similar engineering.

To complete the synthesis of a suitable Taxol precursor, baccatin III, six more hydroxylation reactions and other steps (including some that have not been identified) need to be effectively engineered. Although this is certainly a daunting task, the current study shows potential by providing the basis for the functional expression of two key steps, cyclization and oxygenation, in Taxol biosynthesis. Most importantly, by unlocking the potential of the MEP pathway a new more efficient route to terpenoid biosynthesis is capable of providing potential commercial production of microbially derived terpenoids for use as chemicals and fuels from renewable resources.

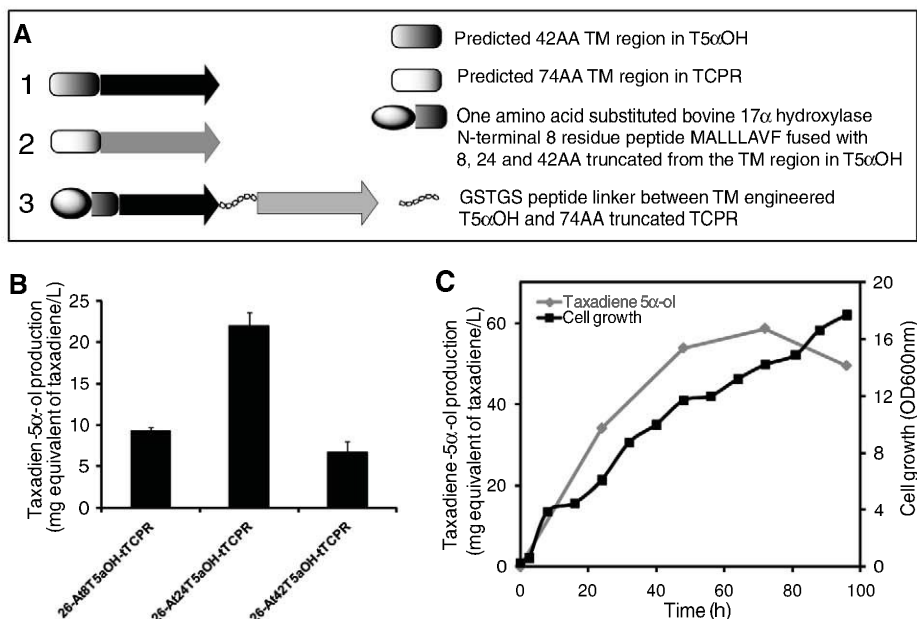


Fig. 4. Engineering Taxol P450 oxidation chemistry in *E. coli*. **(A)** TM engineering and construction of chimera protein from taxadien-5 α -ol hydroxylase (T5 α OH) and *Taxus* cytochrome P450 reductase (TCPR). The labels **1** and **2** represent the full-length proteins of T5 α OH and TCPR identified with 42 and 74 amino acid TM regions, respectively, and **3** represents chimera enzymes generated from three different TM engineered T5 α OH constructs [At8T5 α OH, At24T5 α OH, and At42T5 α OH constructed by fusing an 8-residue synthetic peptide MALLAVF (A) to 8, 24, and 42AA truncated T5 α OH] through a translational fusion with 74AA truncated TCPR (tTCPR) by use of linker peptide GSTGS. **(B)** Functional activity of At8T5 α OH-tTCPR, At24T5 α OH-tTCPR, and At42T5 α OH-tTCPR constructs transformed into taxadiene producing strain 26. Data are mean $\pm$ SD for three replicates. **(C)** Time course profile of taxadien-5 α -ol accumulation and growth profile of the strain 26-At24T5 α OH-tTCPR fermented in a 1-liter bioreactor. Data are mean of two replicate bioreactors.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/330/6000/70/DC1

Materials and Methods

SOM Text

Figs. S1 to S11

Tables S1 to S4

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Reactivity of the Gold/Water Interface During Selective Oxidation Catalysis

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The selective oxidation of alcohols in aqueous phase over supported metal catalysts is facilitated by high-pH conditions. We have studied the mechanism of ethanol and glycerol oxidation to acids over various supported gold and platinum catalysts. Labeling experiments with $^{18}\text{O}_2$ and H_2^{18}O demonstrate that oxygen atoms originating from hydroxide ions instead of molecular oxygen are incorporated into the alcohol during the oxidation reaction. Density functional theory calculations suggest that the reaction path involves both solution-mediated and metal-catalyzed elementary steps. Molecular oxygen is proposed to participate in the catalytic cycle not by dissociation to atomic oxygen but by regenerating hydroxide ions formed via the catalytic decomposition of a peroxide intermediate.

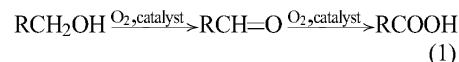
The selective oxidation of alcohols with molecular oxygen over gold (Au) catalysts in liquid water offers a sustainable, environmentally benign alternative to traditional processes that use expensive inorganic oxidants and harmful organic solvents (1, 2). These catalytic transformations are important to the rapidly developing industry based on the conversion of bio-renewable feedstocks to higher-valued chemicals (3, 4) as well as the current production of petrochemicals. Although gold is the noblest of metals (5), the water/Au interface provides a reaction environment that enhances its catalytic performance. We provide here direct evidence for the predominant reaction path during alcohol oxidation at high pH that includes the coupling of both solution-mediated and metal-catalyzed elementary steps.

Alcohol oxidation catalyzed by Pt-group metals has been studied extensively, although the precise

reaction path and extent of O_2 contribution are still under debate (4, 6–8). The mechanism for the selective oxidation of alcohols in liquid water over the Au catalysts remains largely unknown (6, 9), despite a few recent studies with organic solvents (10–12). In general, supported Au nanoparticles are exceptionally good catalysts for the aerobic oxidation of diverse reagents ranging from simple molecules such as CO and H_2 (13) to more complex substrates such as hydrocarbons and alcohols (14). Au catalysts are also substrate-specific, highly selective, stable against metal leaching, and resistant to overoxidation by O_2 (6, 15, 16). The active catalytic species has been suggested to be anionic Au species (17), cationic Au species (18, 19), and neutral Au metal particles (20). Moreover, the size and structure of Au nanoparticles (21, 22) as well as the interface of these particles with the support (23) have also been claimed to be important for catalytic activity. For the well-studied CO oxidation reaction, the presence of water vapor increases the observed rate of the reaction (24–26). Large metallic Au particles and Au metal powder, which are usually considered to be catalytically inert, have consider-

able oxidation activity under aqueous conditions at high pH (27, 28). We provide insights into the active intermediates and the mechanism for alcohol oxidation in aqueous media derived from experimental kinetic studies on the oxidation of glycerol and ethanol with isotopically labeled O_2 and H_2O over supported Au and Pt catalysts, as well as ab initio density functional theory calculations on ethanol oxidation over metal surfaces.

Previous studies indicate that alcohol oxidation over supported metal catalysts (Au, Pt, and Pd) proceeds by dehydrogenation to an aldehyde or ketone intermediate, followed by oxidation to the acid product (Eq. 1)



Hydroxide ions play an important role during oxidation; the product distribution depends on pH, and little or no activity is seen over Au catalysts without added base. We studied Au particles of various sizes (average diameter ranging from 3.5 to 10 nm) on different supports (TiO_2 and C) as catalysts for alcohol oxidation and compared them to Pt and Pd particles supported on C. The oxidation of glycerol ($\text{HOCH}_2\text{CHOHCH}_2\text{OH}$) to glyceric ($\text{HOCH}_2\text{CHOHCOOH}$) and glycolic (HOCH_2COOH) acids occurred at a turnover frequency (TOF) of 6.1 and 4.9 s^{-1} on Au/C and Au/ TiO_2 , respectively, at high pH (>13) whereas the TOF on supported Pt and Pd (1.6 and 2.2 s^{-1} , respectively) was slightly lower at otherwise identical conditions (Table 1). For these Au catalysts, particle size and support composition had negligible effect on the rate or selectivity. In the absence of base, the glycerol oxidation rate was much lower over the Pt and Pd catalysts and no conversion was observed over the Au catalysts (Table 1). Moreover, the products detected over Pt and Pd in the absence of base are primarily the intermediate aldehyde and ketone, rather than acids.

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The effect of initial base concentration on the activity of glycerol oxidation on the supported Pt catalyst was further studied by varying the initial NaOH concentration from 0 to 0.6 M. The TOF for glycerol oxidation over Pt was proportional to the initial hydroxide concentration and thus the initial glycerolate concentration formed by the deprotonation of glycerol [Table 1, fig. S1, and supporting online material (SOM) text], which is consistent with prior work with a supported Au catalyst (29). Although the turnover frequencies

for ethanol ($\text{CH}_3\text{CH}_2\text{OH}$) oxidation to acetic acid (CH_3COOH) over the above-mentioned Au and Pt catalysts were an order of magnitude lower than those observed for glycerol oxidation at identical conditions, Au was still more active than Pt for ethanol oxidation at high pH (table S1).

In an aqueous environment, the initial deprotonation of the alcohol to form an alkoxide intermediate occurs in basic solution, and the extent of the reaction is related to the system pH [the $\text{p}K_a$ (where K_a is the acid dissociation constant) of

alcohols is approximately 16] (30). The initial activation of the alcohol can also occur on the catalyst surface. The calculated activation barriers for the dissociative adsorption of ethanol (Fig. 1A) over the Au(111) and Pt(111) surfaces in liquid water are calculated to be very high at 204 and 116 kJ mol^{-1} , and thus O-H bond activation by the metal alone is unlikely. The presence of surface-bound hydroxide intermediates, however, can facilitate O-H bond activation via proton transfer in much the same way as it occurs in solution. This process lowers the activation barrier to less than 25 kJ mol^{-1} for either metal (Fig. 1B). The presence of adsorbed hydroxide intermediates also lowers the barrier for the subsequent activation of the C-H bond of the ensuing alkoxide intermediate to form the aldehyde over Au. (For Pt, this step already has a very low barrier over the metal without the assistance of adsorbed OH.) The ability of adsorbed hydroxide to activate both the C-H and O-H bonds so effectively on Au(111) helps to explain the overall increase in catalytic activity of the noble metal at high pH.

It is unclear whether O atoms derived from O_2 or from hydroxide ions are involved in carboxylate formation from the intermediate aldehyde. To determine the role of O_2 in the mechanism, the oxidation of aqueous-phase glycerol and ethanol was performed in a batch autoclave reactor using $^{18}\text{O}_2$ in the presence of base (0.3 M glycerol or ethanol and 0.6 M NaOH) together with either Au/C or Au/ TiO_2 (0.8 and 1.6% Au, respectively, obtained from the World Gold Council) as a catalyst (31). The isotopomer distribution in the product was evaluated by mass spectrometry. Glyceric acid was the major product of glycerol oxidation for all catalysts evaluated at high pH (Table 1). However, no discernable amount of labeled O was observed in the glyceric acid product. Figure 2 shows a representative reaction profile for the oxidation of glycerol as well as the mass spectrum of the product glyceric acid. For the reaction performed with $^{18}\text{O}_2$, only one peak in the mass spectrum of glyceric acid corresponding to the unlabeled product was observed (fig. S2). Analogous experiments with ethanol oxidation confirmed a lack of ^{18}O incorporation into the product acetic acid (fig. S5). Regardless of the Au particle size, the composition of the support, and the structure of the alcohol, ^{18}O was not observed in the products of alcohol oxidation.

The lack of ^{18}O incorporation in the acid products might be caused by an inability of Au nanoparticles to dissociatively adsorb O_2 (14, 32, 33). We studied glycerol oxidation over C-supported Pt (1%, obtained from Sigma-Aldrich) and Pd (3%, Sigma-Aldrich) with $^{18}\text{O}_2$ at high pH (31), because these transition metals dissociate O_2 (34). However, no labeled O was observed in the glyceric acid product (fig. S2, B and C). Because base is not required to oxidize alcohols over Pt, glycerol oxidation was carried out with $^{18}\text{O}_2$ over Pt/C in neutral water. Again, no ^{18}O -labeled acid products were observed in any appreciable amounts (fig. S2D), which is con-

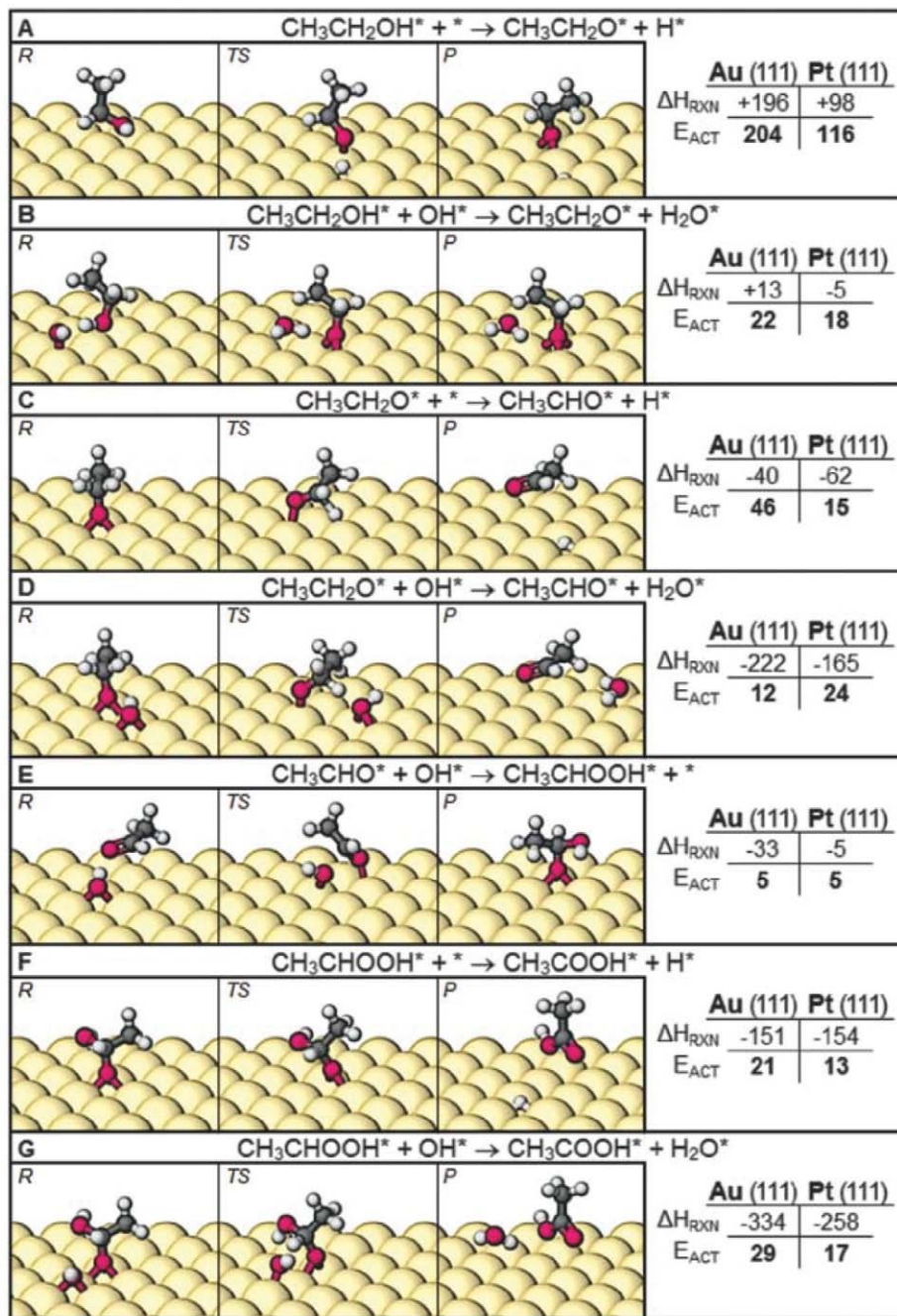


Fig. 1. (A to G) Selected reaction energies and activation barriers (in kilojoules per mole) for the oxidation of ethanol to acetic acid on Au(111) and Pt(111) surfaces in the presence of liquid water. The reactant (R), transition state (TS), and product (P) on the surfaces were computed with density functional theory. The solution-phase water was omitted for clarity.

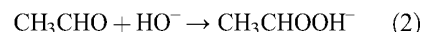
sistent with prior work involving ethanol oxidation over Pt in neutral solution (35). To confirm that the acid products do not exchange O appreciably with liquid water, the major products from glycerol and ethanol oxidation (glyceric, glycolic, and acetic acid) were dissolved separately in H_2^{18}O and heated to the reaction conditions in the presence of base and a Au catalyst for the duration of a typical reaction test (3 hours). No appreciable incorporation of ^{18}O from labeled water was observed in the products (figs. S2 to S5). It should be emphasized that no glycerol oxidation was observed in the absence of O_2 .

To explore the role of hydroxide in Au-catalyzed oxidation, glycerol and ethanol were oxidized over Au/C and Au/TiO₂ with labeled water

(H_2^{18}O) at high pH (0.3 M glycerol or ethanol and 0.6 M NaOH) and $^{16}\text{O}_2$ as the gas-phase oxidant. The mass spectrum for glyceric acid produced during glycerol oxidation (Fig. 2 and fig. S2) shows that a range from one to four ^{18}O atoms was incorporated into the glyceric acid product. Incorporation of multiple labeled ^{18}O atoms into the acetic acid product of ethanol oxidation over Au/C and Au/TiO₂ (fig. S5) and into the minor oxidation products of glycerol was also observed (figs. S3 and S4). Because the reaction products do not exchange O with water on the time scale of the reaction, the appearance of ^{18}O in the product during reactions performed in H_2^{18}O suggests that aqueous-phase alcohol oxidation proceeds through an alkoxy intermediate

of the geminal diol that was formed by the reaction of the aldehyde intermediate with hydroxide (Figs. 1E and 3). Rapid exchange of the diols with $^{18}\text{OH}^-$ accounts for the two ^{18}O atoms found in the acetic acid product from ethanol oxidation. During glycerol oxidation, glyceraldehyde ($\text{HOCH}_2\text{CHOHCHO}$) is the reaction intermediate produced by the initial dehydrogenation of the terminal alcohol of glycerol. The base-catalyzed rapid interconversion of glyceraldehyde to dihydroxyacetone ($\text{HOCH}_2\text{COCH}_2\text{OH}$) (DHA) during the oxidation reaction could account for more than two ^{18}O atoms appearing in the product (SOM text) (36). Indeed, a control experiment with glyceraldehyde (0.05 M) in the presence of Au/C and a small amount of base (0.01 M NaOH to avoid degradation of glyceraldehyde) in H_2^{18}O resulted in ^{18}O incorporation in DHA that was formed during the experiment, as seen in fig. S6.

Theory was used to examine the base-catalyzed aqueous-phase oxidation of acetaldehyde (CH_3CHO) in solution, as shown in Eq. 2, and to explore the role of hydroxide ion as the oxygen source



The activation barrier for this reaction in solution was calculated to be 45 kJ mol^{-1} . The barrier is largely due to the energy required to restructure the H-bonding water network around the solvated hydroxide anion and the aldehyde to allow them to react together. The same reaction was also examined on the Au(111) and Pt(111) surfaces in the presence of solvent, which resulted in activation barriers of 5 kJ mol^{-1} for both metals (Fig. 1E).

Table 1. Glycerol oxidation over Au/C, Au/TiO₂, Pt/C, and Pd/C in liquid water.

Catalyst	NaOH: glycerol (mol:mol)	TOF (s^{-1})	Conversion (%)	Selectivity (%)				
				Glyceric acid	Glycolic acid	Tartronic acid	Glyceraldehyde	Dihydroxyacetone
Au/C*	2.0	6.1	6.8	67	33	0.0	0.0	0.0
Au/TiO ₂ *	2.0	4.9	33	64	24	2.0	0.0	0.0
Pd/C*	2.0	2.2	29	83	6.0	5.0	0.0	0.0
Pt/C*	2.0	1.6	16	70	21	7.0	0.0	0.0
Pt/C*	1.0	0.76	7.1	78	14	7.0	0.0	0.0
Pt/C*	0.5	0.48	4.7	72	28	0.0	0.0	0.0
Pt/C†	3×10^{-4}	0.05	8.0	29	0.0	0.0	50	21
Pt/C†	0.0	0.06	9.0	25	0.0	0.0	54	21
Pd/C†	0.0	0.004	1.3	25	0.0	0.0	50	25
Au/C†	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Au/TiO ₂ †	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

*Reaction conditions: 0.3 M glycerol (G); 30 ml; at 333 K; $\text{PO}_2 = 11$ bar; G:Au = G:Pt = 8000 (mol:mol); G:Pd = 2500 (mol:mol); time (t) = 0.5 hours.

†Reaction conditions: 0.3 M glycerol; 5 ml; at 333 K; $\text{PO}_2 = 11$ bar; G:Au = G:Pt = 5000 (mol:mol); G:Pd = 2000 (mol:mol); t = 5 hours. Gold particle sizes: Au/C = 10.5 nm, Au/TiO₂ = 3.5 nm. Dispersion (estimated ratio of surface to total metal atoms): Au/C = 0.05, Au/TiO₂ = 0.29, Pt/C = 0.43, Pd/C = 0.33 (32).

Fig. 2. Reaction profile for the oxidation of glycerol over Au/C in liquid water. Reaction conditions were as follows: 0.3 M glycerol; NaOH: glycerol = 2.0 (mol:mol); glycerol: Au = 8000:1 (mol:mol); at a temperature of 333 K; with a partial pressure of O_2 (PO_2) = 11 bar. (Inset at bottom) LC mass spectrum (electronegative ion mode) of glyceric acid (molecular weight 106) formed during glycerol oxidation over Au/C in $^{18}\text{O}_2 + \text{H}_2^{16}\text{O}$ or $^{16}\text{O}_2 + \text{H}_2^{18}\text{O}$. Glycerol oxidation in $^{16}\text{O}_2 + \text{H}_2^{18}\text{O}$ results in the incorporation of multiple ^{18}O atoms in the product glyceric acid. m/z , mass/charge ratio.

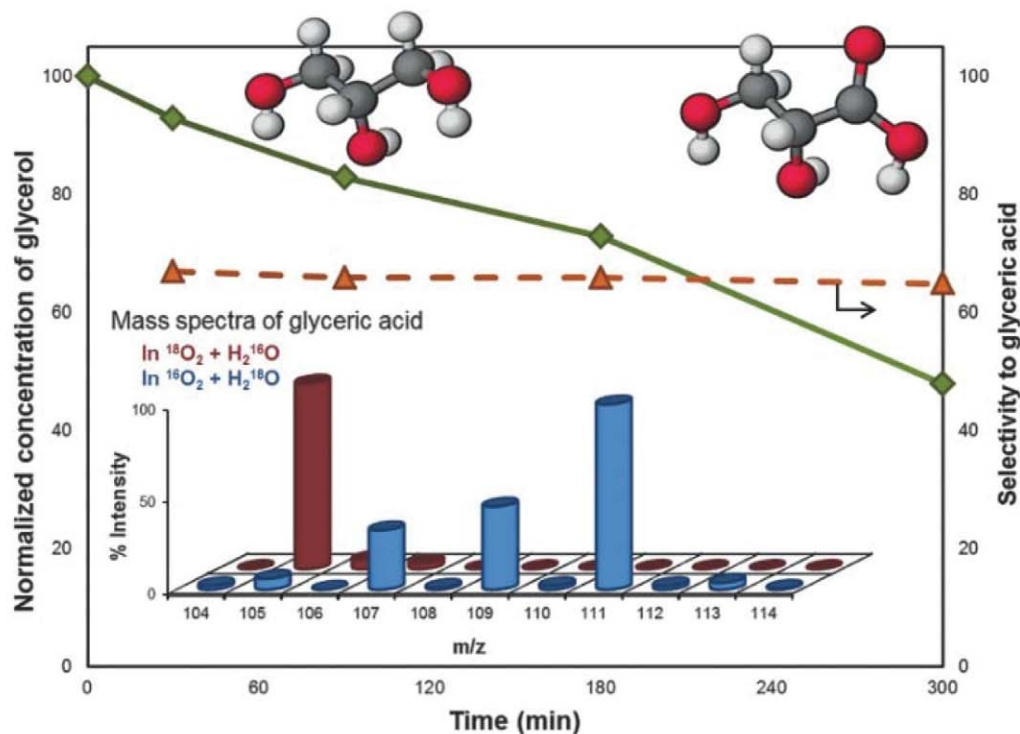
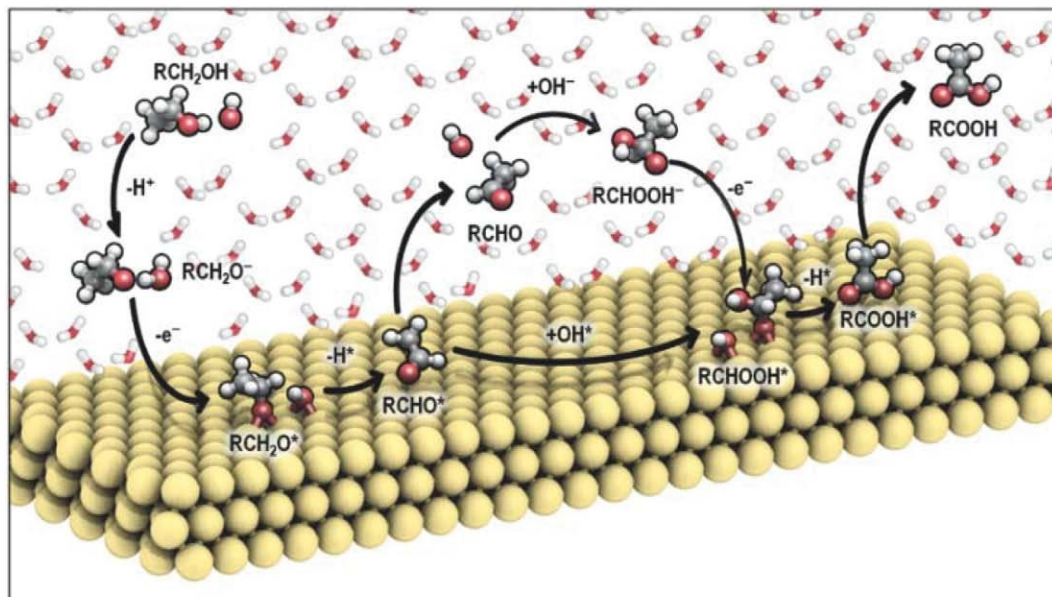


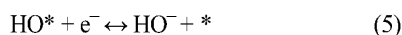
Fig. 3. Reaction scheme for the oxidation of alcohols to acids over a Au surface in water at high pH. Hydroxide facilitates elementary steps in alcohol oxidation in both the solution phase and at the metal/solution interface.



These results account for the increase in reactivity with HO^- either in solution or at the solution/metal interface. The subsequent C-H activation of the $\text{CH}_3\text{CHOOH}^-$ intermediate to form acetic acid occurs quite readily over Pt as well as Au, with barriers of only 10 to 20 kJ mol^{-1} (Fig. 1F).

Several roles for O_2 during the oxidation reactions in water at high pH have been proposed, including the direct participation of atomic O during dehydrogenation reactions, the reduction of atomic O to water through H atoms adsorbed on the surface, the direct oxidation of the intermediate aldehyde with atomic O to form the O-insertion product acids, and the removal of strongly bound organic adsorbates such as CO through oxidation (6). All of these routes require O_2 dissociation on the catalyst surface, which is not favored on Au catalysts (14, 32, 33) and may be inhibited by water or hydroxides that adsorb on Pt-group catalysts. The activation barriers for O_2 dissociation on Au(111) and Pt(111) were calculated to be 105 and 64 kJ mol^{-1} , respectively, confirming the noble nature of Au as compared to Pt-group metals (table S3, reaction 7).

The production of peroxide during oxidation reactions over Au (28, 29) indicates that O_2 is first reduced during these reactions before dissociation. [For the case of Pt, O_2 dissociation is also difficult because of the high surface coverages of hydroxide intermediates (8).] Activation of O_2 occurs through the formation and dissociation of peroxide (OOH^*) and hydrogen peroxide (HOOH^*) intermediates (Eqs. 3 to 5) analogous to those formed electrocatalytically under alkaline conditions via four-electron or two-electron processes (37)



where * represents a site on the metal surface. This sequence accounts for the removal of electrons added to the surface during the adsorption of hydroxide ions and the regeneration of HO^- species via O_2 reduction with water, thus closing the catalytic cycle. The steady-state coverage of hydroxide on the surface is probably limited by the ability of the metal to accommodate the excess negative charge.

To test the feasibility of this reduction sequence, density functional theory calculations were carried out over both Au and Pt surfaces. Reduction of O_2 by water had a low barrier of 16 kJ mol^{-1} on Au(111). The peroxide that forms can subsequently dissociate on Au to form atomic O and hydroxide, with a barrier of 83 kJ mol^{-1} ; however, the more likely step is the further reduction of OOH^* to H_2O_2^* , with a barrier of only 48 kJ mol^{-1} (table S3). The decomposition of H_2O_2^* to hydroxide has an activation barrier of 71 kJ mol^{-1} . The decomposition of hydrogen peroxide over Pt and Pd has a barrier of only 29 and 5 kJ mol^{-1} , respectively. The low calculated barrier for peroxide decomposition on Pd as compared to Au explains why Ketchie *et al.* observed appreciable concentrations of peroxide in the product mixture formed during glycerol oxidation over Au/C, whereas only trace amounts of peroxide were detected when Pd/C was used as a catalyst at identical reaction conditions (38). Our computational results, coupled with the isotopic labeling studies, demonstrate that the role of O_2 during alcohol oxidation is an indirect one that does not involve incorporation into the acid products but instead regenerates hydroxide ions.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/330/6000/74/DC1
Materials and Methods
SOM Text
Figs. S1 to S8
Scheme S1
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Human Adaptation and Plant Use in Highland New Guinea 49,000 to 44,000 Years Ago

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After their emergence by 200,000 years before the present in Africa, modern humans colonized the globe, reaching Australia and New Guinea by 40,000 to 50,000 years ago. Understanding how humans lived and adapted to the range of environments in these areas has been difficult because well-preserved settlements are scarce. Data from the New Guinea Highlands (at an elevation of ~2000 meters) demonstrate the exploitation of the endemic nut *Pandanus* and yams in archaeological sites dated to 49,000 to 36,000 years ago, which are among the oldest human sites in this region. The sites also contain stone tools thought to be used to remove trees, which suggests that the early inhabitants cleared forest patches to promote the growth of useful plants.

Sahul, the single Pleistocene continent linking New Guinea, Australia, and Tasmania, is thought to have been colonized by humans some time after 50,000 years ago (*1*). Reaching Sahul required crossing water from Southeast Asia. Most early sites in New Guinea have been found along the coastal margins (Fig. 1, inset; fig S1; and table S2). An exception first documented in the 1960s is an open site [Papua New Guinea (PNG) National Museum site code AER] in the Ivane Valley of the New Guinea Highlands, where excavations at the Kosipe Mission recovered stone artefacts including waisted tools dated then to 26,870 ± 590 ¹⁴C years before the present (radiocarbon lab no. ANU-191) (calibrated to 30,350 to 32,580 years ago) (*2*). Kosipe Mission is located at ~2000 m above sea level, on the spur of a hill overlooking a large swamp (Fig. 1). Further

excavations in 2005 (*3*) extended known occupation there to 41,000 to 38,000 years ago (table S1). A carbonized kernel of a pandanus nut was recovered in the 36,000- to 34,000-year-old levels.

Subsequent fieldwork in 2007 and 2008 has identified late Pleistocene to Holocene occupation at seven additional locations across the Ivane Valley, with the earliest site dating to between 49,000 and 43,000 calibrated years before the present (table S1). Here we describe the evidence for early occupation of these sites and the subsistence strategies employed by these early colonists.

Sediments in the Ivane valley are dominated by a series of volcanic tephra that are probably derived from Mount Lamington some 140 km to the southeast. All eight highland sites have a basic set of five identified layers: a dark brown topsoil (layer 1), a brown-orange clay (layer 2); a black-brown soil (layers 3a and 3b), and a gray soil (layer 4). These occupation layers overlay culturally sterile orange clay (layer 5). Layer 3 is separated into two distinct units (a and b) at Vilakuav (*2*), representing separate ash falls. We subsequently identified these two layers (3a and 3b) in most sites. At Vilakuav, Joe's Garden, and Kosipe Mission, they are separated by a thin band of charcoal (fig. S2). Accelerator mass spectrometry dates were derived from charcoal collected in situ. We use the calibrated dates (*4*) in our discussion. All dates from Joe's Garden, Vilakuav, South Kov, and Airport Mound are presented here for the first time.

The earliest dates for occupation of the valley are at Vilakuav, between 49,000 and 43,000 years ago; and three sites (Vilakuav, South Kov, and Airport Mound) contain artefacts that yield calibrated ¹⁴C dates earlier than 42,500 years ago, at 95.4% confidence levels. Occupation at the Kosipe Mission site is dated to 41,400 to 38,000 years ago, again at a 95.4% confidence level. Layer 3b shows occupation from 38,500 to 30,000 years ago, and layer 3a dates from 30,000 to 26,000 years ago. Layer 2 dates to the Holocene (table S1).

Our radiocarbon ages are some of the oldest dates for any Sahul site (table S2), excluding several contentious 20-year-old claims in Australia (*1*). Together with indirectly dated artefacts from Bobongara on the Huon Peninsula, the oldest occupational layers at the highland sites of Vilakuav, Airport Mound, and South Kov are older than any other known sites in New Guinea or Island Melanesia.

All highland sites lack evidence of any occupation during the Last Glacial Maximum (LGM). The mean temperature of the coldest month at Kosipe at the LGM has been estimated to have been between 6.3° to 9.2°C colder than today (*5, 6*). The climates before the LGM would also have been cooler than those today. Layers 4 and 3b from the Ivane Valley correspond to marine isotope stage (MIS) 3, whereas layer 3a corresponds with the shift to much colder conditions from MIS 3 to MIS 2 (*7*). Other palynological evidence from the Ivane Valley (*6*) also indicates that the tree line was lower during the deposition of layers 4 and 3 and that the vegetation zones were compressed downward during the earliest occupation, which is consistent with a colder climate.

Stone artefacts were recovered from the earliest levels (layer 4) of Kosipe Mission, Airstrip Mound, South Kov, and Vilakuav (Fig. 2). Artefacts are made from a diverse range of raw materials, including basalt, schist, baked siliceous metasediment, dolerite, metabasalt, and quartz. Of these, baked siliceous sediment is the only rock type not found in the Ivane Valley. Samples of this rock were obtained along the Kosipe-Woitape track above Woitape, ~20 km distant. It is valuable for making tools because it flakes easily yet is hard.

Two waisted stone artefacts made from schist and metabasalt were found from layer 4 at South Kov and one from layer 4 at Airstrip Mound (Fig. 2 and fig. S3). This distinctive ar-

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tefact type was also found in layer 3a at South Kov and in layer 3b at Joe's Garden (Fig. 2 and fig. S4) and Vilakuav, but disappears before the LGM. Frequently referred to as waisted axes, they also occur in several other Pleistocene highland and lowland sites of the New Guinea mainland. Waisted axes have been seen as implements used to modify forest environments by opening up patches to sunlight to promote the growth of food and other useful plants (8). In the Ivane Valley, other Pleistocene stone artefacts include flaked artefacts, tool blanks, axe-like bifacially flaked implements, and flaking debris. An expanded range of stone artefacts occurs in the Holocene layers at all other sites except for Airport Mound.

Onsite stone artefact manufacture at all sites is indicated by the presence of cores and manufacturing debris and the use of local raw materials. River cobble cortex on both igneous and metamorphic artefacts suggests that local waterways were the primary source of raw material. The stone assemblage is consistent with finds from Bobongara, the only other mainland late Pleistocene assemblage older than 35,000 years.

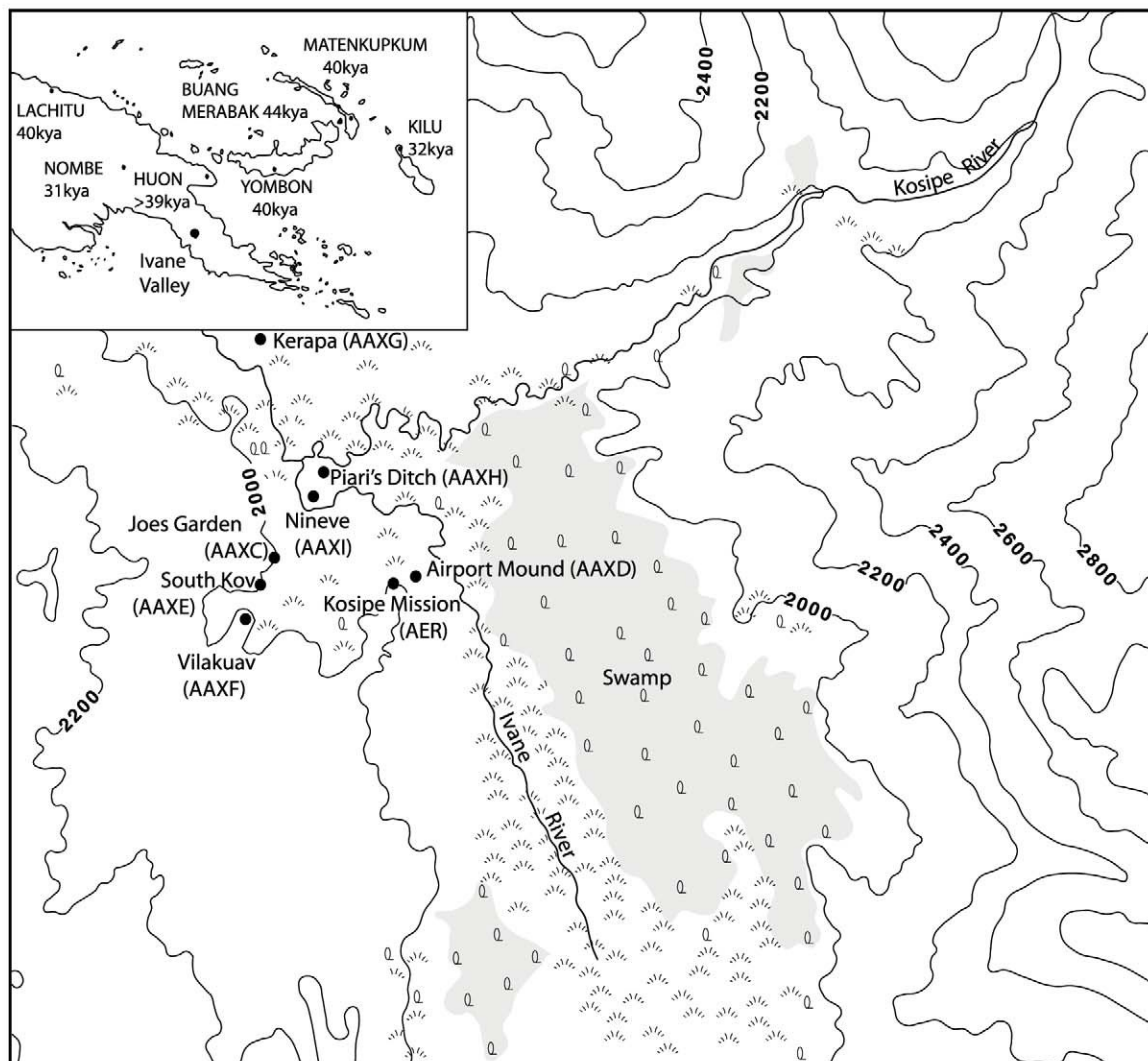
At this site, waisted artefacts were recovered below tephra 2 which dates to $38,000 \pm 600$ years by thermoluminescence (TL) dating (9), although the excavators argue for an estimate of 40,000 years or more, based on high moisture levels affecting the TL readings.

Abundant starch grains were extracted from several of the stone artefacts from layer 3a at Joe's Garden and layer 4 at the South Kov site (table S2). Several of these were consistent with starch from yams, specifically *Dioscorea* species. The size and morphology of the tuber-like starch grains (figs. S5 and S6) from the Joe's Garden samples were consistent with *Dioscorea alata* (Fig. 3) comparative reference material. The sample sizes of the yam starch grains from both South Kov artefacts were smaller ($n = 7$ and $n = 8$ grains), although two starch grains exceeded $40 \mu\text{m}$ in size. The shape and surface features of these starches are unlike that of *D. bulbifera* but broadly similar to those of *D. alata* (fig. S6C) and/or *D. pentaphylla* (fig. S6D). There is no clear sculpting of the hilum end of the grain in fig. S6C or S6D, as seen in most grains from *D. pentaphylla*. However, this feature is not

present in all *D. pentaphylla* grains, and therefore we cannot exclude a contribution from this species.

Charred *Pandanus* nutshells were identified in Kosipe Mission layer 3, layers 3 and 4 from Joe's Garden, and all layers from South Kov and Vilakuav. All specimens, including fragments, were from single-seeded species of highland *Pandanus* in the section *Karuka* (10), which contain nutritious seeds. Morphometric analysis confirmed that complete specimens from South Kov layers 3 and 4 were not from the economically important *Pandanus brosimos* or *P. julianetii*, being most similar to an unclassified foraged wild species known as *Taip* (see supporting online material). A human source for the archaeological remains is indicated by (i) a close repeated association with charcoal, bone fragments, and tools and (ii) the absence of rodent/cuscus gnaw marks on the seeds. The absence of nuts from some sites and layers may indicate variability in activity areas across the landscape. *Pandanus* was absent at the Airport Mound site and in samples from the swamp sections taken for palynological analysis (6).

Fig. 1. Map of the Ivane valley with archaeological sites. Seven new late Pleistocene occupation sites were identified. Five are situated on spurs above the valley floor, ranging in altitude from 2020 to 1940 m: Joe's Garden, South Kov, Airport Mound, Kerapa, and Vilakuav. Two sites were located on the western valley floor: Piari's Ditch and Nineve.



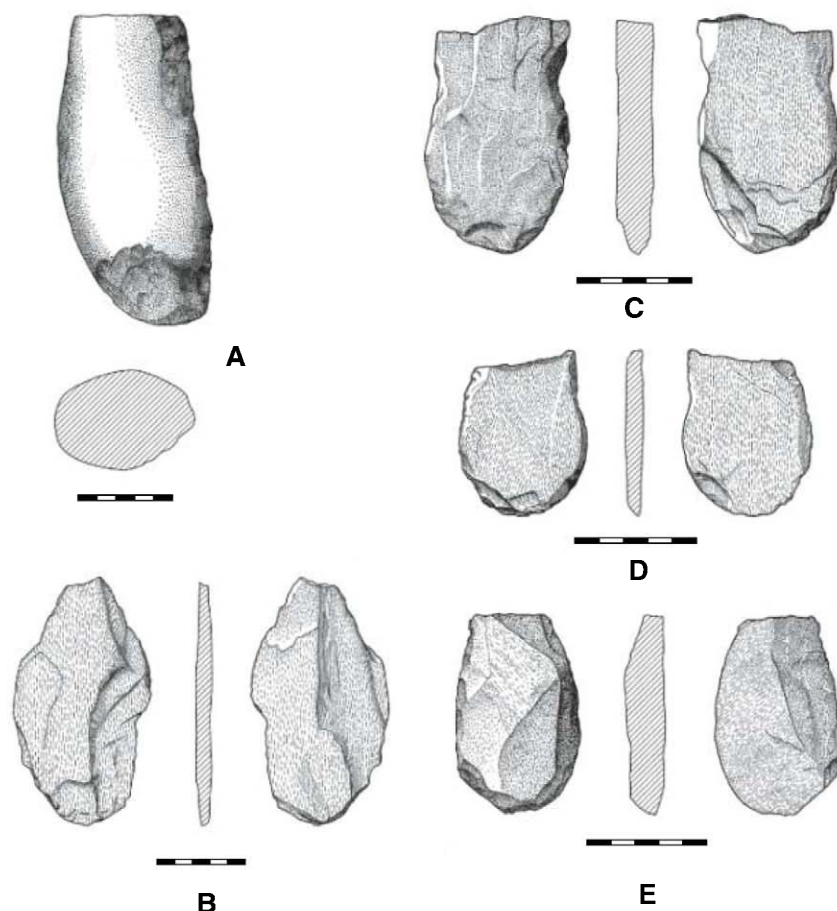
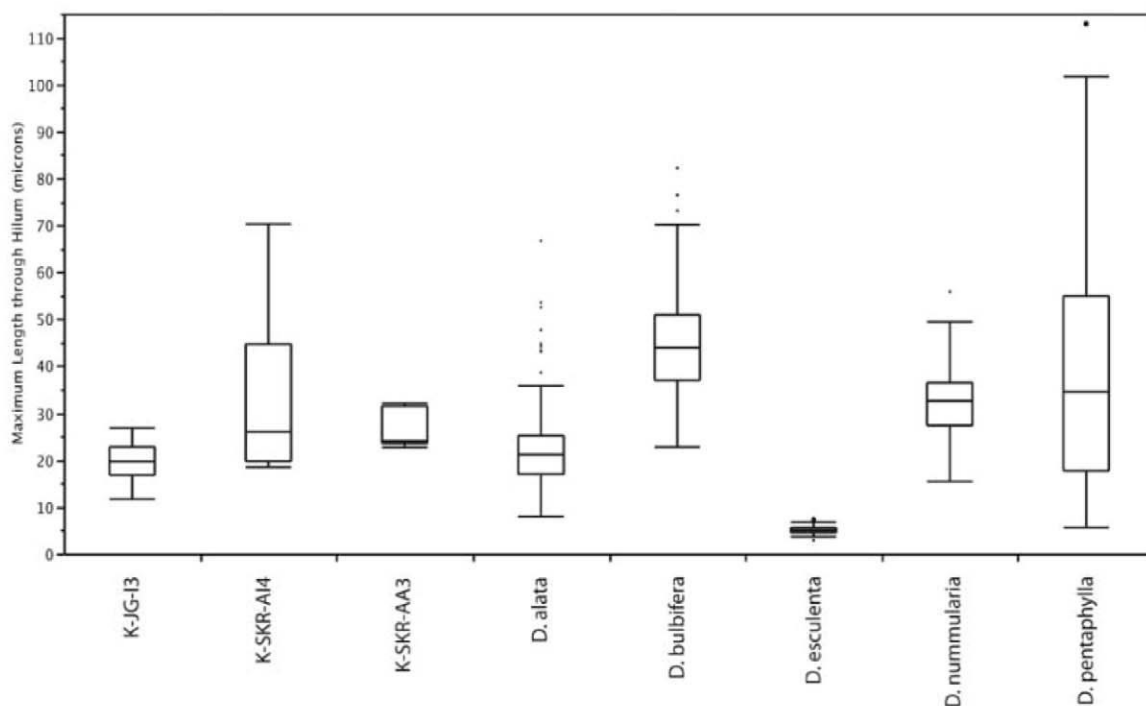


Fig. 2. Ivane Valley late Pleistocene stone tools. (A and B) Airstrip Mound (PNG National Museum site code AAXD) from layer 4. (C) Joe's Garden (AAXC) from layer 3b. (D and E) South Kov (AAXE) from layer 4. Scale bars are in centimeters.

Fig. 3. Box plots of the maximum dimensions of starch grains through the hilum in micrometers. The box includes 50% of the population, and the horizontal line represents the median value. The tuber grains presented from each archaeological preparation are only a small portion of the total counts from each artefact. The K-JG-13 sample matches entirely with the *D. alata* sample. For the K-SKR-A14 sample, the variation in grain length indicates that it is likely to represent more than one type of yam. The third artefact, K-SKR-AA3, comprises only seven grains, and these overlap with all samples except the *D. esculenta* sample.



The preservation of food plant species in open sites of this age is remarkable, and the analysis confirms that *Pandanus* and yams were used for subsistence in this valley from the time that the earliest colonists arrived. Although *Pandanus* would have grown abundantly in the local environment, yams would have been found at lower altitudes, indicating that gathering territories included lower altitudinal zones beyond the Ivane Valley. The gathering of food plants was accompanied by hunting of small animals. Burnt highly fragmented bone was recovered from the lowest levels at Vilakuav (1.1 g from layer 4 and 0.3 g from layer 3b), although it has been impossible to identify the species hunted. The acidic soil ensures that only the highly fragmented burnt bone survives. Although we have little evidence for the nature of hunted animals in the Ivane Valley diet, the range of potential high-altitude game in New Guinea is wide and well documented from later Highlands sites farther to the west (11).

The nature of human impact on the local environment is difficult to assess. In the Ivane Valley, Hope (6) noted an increase in microscopic charcoal between 41,000 and 38,000 calibrated years before the present, soon after the Kosipe Zone-2 stage (KOS-2) (at 42,000 years ago). Hope argued that the early humans burned the wet montane vegetation. The activities of people were probably a contributing factor to the physical changes to the Ivane basin. The base of layer 4 is indicative of the presence of a swampy lake (Hope's KOS-4 phase), which slowly developed into a peat swamp that extended to the south by the time of occupation in layer 3b (6). A human presence, increased fire, and changes in the lake hydrology are likely to be related.

Our data show that people occupied a New Guinea valley at 2000 m above sea level soon after their arrival in Sahul (1). As the climate cooled, the optimal growing conditions for yams would have occurred at lower altitudes. This may indicate that *Pandanus* was the most important staple at this time and help explain the late Pleistocene abandonment of the highland sites. Foraging into this high-altitude environment would guarantee a high return in plant fat and protein to complement local animal foods, the starch-rich yams from lower altitudes, and those foods not preserved in the archaeological record.

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12. This research was supported by the Marsden Fund Council from government funding administered by the Royal Society of New Zealand. We thank G. Hope for introducing us to the Ivane Valley and companionship in the field; the communities of the Ivane Valley for their support; and the National Research Institute of PNG and the National Museum and Art Gallery of PNG for their support and affiliation. The authors acknowledge

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Supporting Online Material

www.sciencemag.org/cgi/content/full/330/6000/78/DC1
SOM Text
Figs. S1 to S9
Tables S1 to S3
References

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Identifying the Driver of Pulsating Aurora

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Pulsating aurora, a spectacular emission that appears as blinking of the upper atmosphere in the polar regions, is known to be excited by modulated, downward-streaming electrons. Despite its distinctive feature, identifying the driver of the electron precipitation has been a long-standing problem. Using coordinated satellite and ground-based all-sky imager observations from the THEMIS mission, we provide direct evidence that a naturally occurring electromagnetic wave, lower-band chorus, can drive pulsating aurora. Because the waves at a given equatorial location in space correlate with a single pulsating auroral patch in the upper atmosphere, our findings can also be used to constrain magnetic field models with much higher accuracy than has previously been possible.

The aurora is a spectacular natural phenomenon that occurs in Earth's polar regions, exhibiting a range of scale sizes (~1 to 100 km) and characteristic wavelengths (e.g., 427.8, 557.7, and 630.0 nm) (1, 2). Auroral features are a visual display of the patterns of energetic particles from distant regions in Earth's magnetosphere that move along magnetic field lines, causing photon emissions in the upper atmosphere. This process represents an important loss of energetic particles from the magnetosphere, and the energy

from these particles causes drastic changes in ionization of the upper atmosphere. One type of aurora, the pulsating aurora (PA), has attracted much attention because of its distinctive luminosity patches at ~100-km altitude, which have a horizontal scale size of ~100 km and switch on and off with recurrence periods of ~5 to 40 s (3, 4).

Rocket and low-altitude spacecraft observations have revealed that auroral pulsations result from a time-varying flux of precipitating electrons with energies exceeding ~10 keV (3, 5). However, the driver of this precipitation has not been uniquely identified. Theoretical investigations have shown that resonant interactions with naturally occurring emissions, known as chorus, could lead to the precipitation of energetic electrons in the appropriate energy range for PA (4, 6), but this suggestion has been difficult to verify experimentally. Chorus consists of discrete bursts of wave power that are confined near the magnetic equator (7, 8) and typically occur in distinct lower- and upper-frequency bands, below and above half of the equatorial electron cyclotron frequency (f_c). Lower- and upper-band chorus can resonate with more than 10 and less than a few keV electrons (9), respectively. In addition, chorus may evolve nonlinearly for large ampli-

tudes (10) and is frequently associated with electrostatic cyclotron harmonic (ECH) waves, which occur above $1 f_c$ and can resonate with electrons of energies below a few keV (11).

Attempts have been made to test wave theories using low-altitude rocket observations (12) and high-altitude, equatorial spacecraft (13–15). Although a general correspondence between PA and electromagnetic waves has been observed, a one-to-one correlation between individual bursts of chorus and auroral pulses has been elusive. This discrepancy between observation and theoretical prediction has been attributed to the difficulty in finding the exact mapping of a distant spacecraft to a small (~100 km) pulsating auroral patch in the upper atmosphere along magnetic field lines. Such mapping is particularly difficult because adjacent auroral patches typically pulsate independently of each other (16). Additionally, the simultaneous existence of multiple magnetospheric plasma waves (13, 17) makes it difficult to identify the specific wave mode responsible for electron scattering leading to PA.

Here, we report observations of PA obtained on 15 February 2009 using one of the ground-based All-Sky Imagers (ASIs) (18) of the THEMIS mission (19). Their broad latitudinal and longitudinal coverage (~1000 km), as well as high resolution (~1 km spatial and 3 s temporal), allowed us to observe the evolution of localized, individual pulsating auroral patches. We also report on plasma wave observations made in space by THEMIS-A (20–22). During the period of observation, this spacecraft was located near the magnetic equator in the Southern Hemisphere, and the magnetic field line threading the spacecraft was located close to the center of the imager field of view, which avoids optical distortion.

In the model shown in Fig. 1A, electrons that are initially trapped by Earth's magnetic field encounter chorus propagating away from the equator. The electrons are scattered and precipitate into the upper atmosphere, resulting in

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the auroral light observed by the ASI. A typical lower-band chorus spectrum at frequencies between 0.05 and $0.3 f_c$ (Fig. 1B) shows repetitive discrete bursts every ~ 10 s, which would be composed of multiple chorus elements. Intense lower-band chorus was present during this time period, whereas both upper-band chorus (0.5 to $0.8 f_c$) and ECH ($>1.0 f_c$) waves were not observed, even though the spacecraft was located close to the equator.

Images from the Narsarsuaq (Greenland) ASI (Fig. 1C) at the four selected times (see movie S1 for the entire image sequence) display discrete aurora, which is the bright, structured emission in the northern portion, as well as fainter, unstructured emissions to the south, called diffuse aurora. Auroral patches with a scale size of ~ 100 km pulsating at $\sim 64^\circ$ to 67° magnetic latitude (MLAT) embedded in the diffuse aurora are PA; their repetitive intensity modulation is seen clearly in movie S1. The images in the second and fourth

panels in Fig. 1C were obtained simultaneously with intense chorus shown in Fig. 1B (vertical lines b and d). A bright auroral patch to the west of the model footprint of the spacecraft is not present in the first and third panels. Adjacent auroral patches do not pulsate in phase with the chorus intensity modulation, implying a correlation between the chorus emission measured at the spacecraft and the pulsating auroral patch. It is thus likely that the auroral patch that best correlates with the chorus intensity modulation pinpoints the source of the precipitation in space and the correct footprint of the spacecraft rather than the model footprint shown in Fig. 1C.

To investigate this hypothesis, we calculated cross-correlation coefficients between the lower-band chorus intensity and auroral luminosity in each imager pixel using the entire period of the wave observations shown in Fig. 1B. The auroral pulsations have an almost one-to-one correspondence with each burst of chorus (Fig. 1D). The

high correlation (0.88 , $SE = 0.13$) supports our inference that intensity-modulated lower-band chorus was driving this PA. Although the time lag between these two wave forms was considered, we found that the simultaneous correlation was much higher. This is consistent with the short travel times (<1 s) of >10 keV precipitating equatorial electrons down the field line causing PA, and the short lifetime (~ 1 s) of the excited atmospheric atoms (23). Both of these time scales fall within the time resolution of the imager (3 s).

We also calculated spatial distributions of the cross-correlation coefficient for all imager pixels. The first image of Fig. 2B reveals a well-grouped patch of high correlation. The correlation coefficient outside this patch diminishes rapidly with distance, despite the presence of several other pulsating auroral patches located within the imager field of view. The high-correlation region demarcates the auroral patch marked in panels b and d of Fig. 1C, highlighting that this is the only

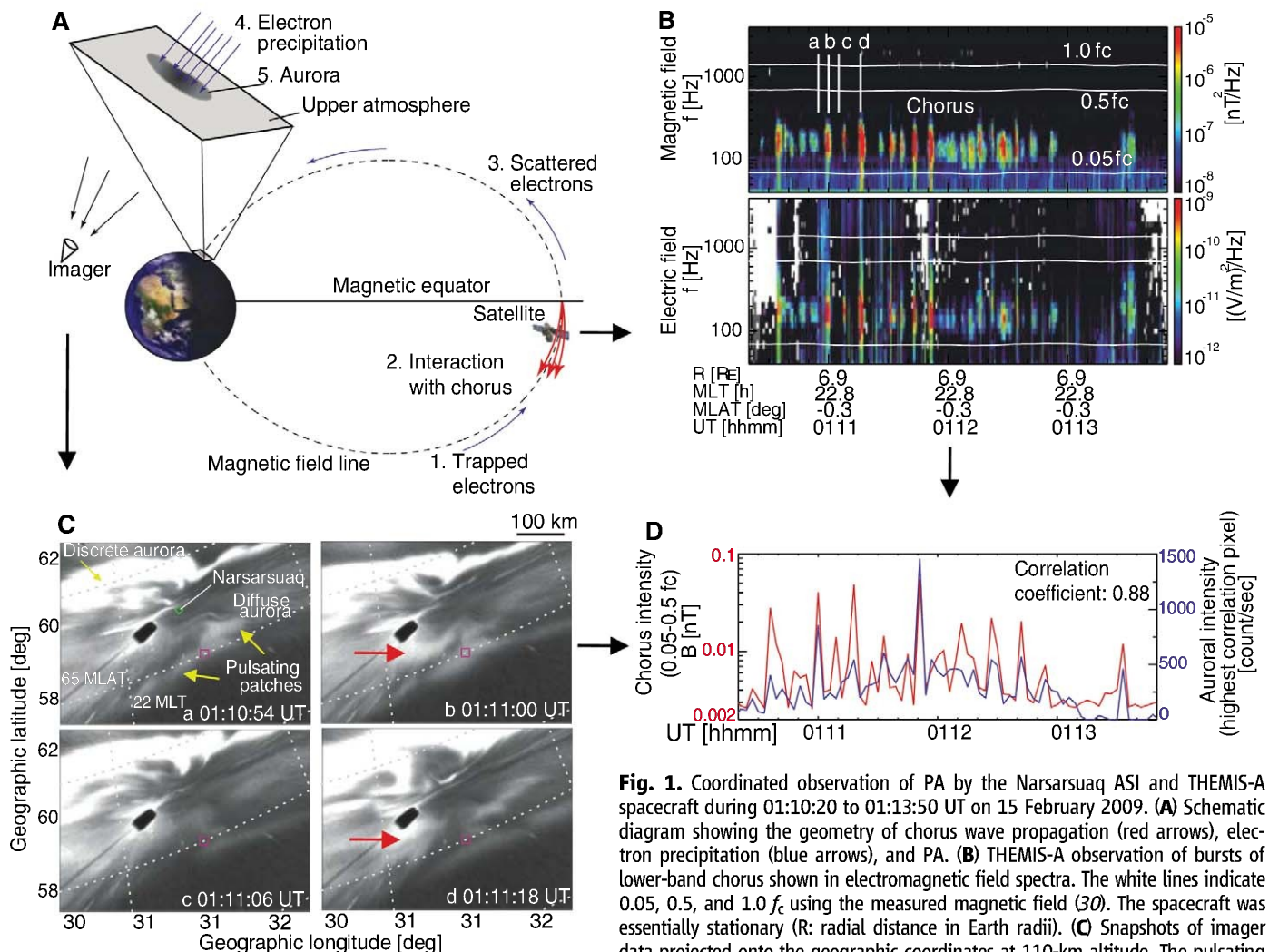


Fig. 1. Coordinated observation of PA by the Narsarsuaq ASI and THEMIS-A spacecraft during 01:10:20 to 01:13:50 UT on 15 February 2009. **(A)** Schematic diagram showing the geometry of chorus wave propagation (red arrows), electron precipitation (blue arrows), and PA. **(B)** THEMIS-A observation of bursts of lower-band chorus shown in electromagnetic field spectra. The white lines indicate 0.05 , 0.5 , and $1.0 f_c$ using the measured magnetic field (30). The spacecraft was essentially stationary (R : radial distance in Earth radii). **(C)** Snapshots of imager data projected onto the geographic coordinates at 110-km altitude. The pulsating patch correlating with chorus is indicated by the red arrows. ASI snapshot times are also marked in **(B)** by white vertical lines. The pink square shows the magnetic footprint of the THEMIS-A spacecraft using the Tsyganenko 96 (31) magnetic field model (the model was used only for a rough estimation of the footprint, and the choice of the model is arbitrary). The spacecraft footprint was located close to the center of the imager field of view (green square in panel a). Dashed lines give magnetic coordinates every 3° in latitude and 1 hour in local time. The black spot near the center of each image is an artificial object. **(D)** Correlation of lower-band chorus integrated magnetic field intensity over 0.05 to $0.5 f_c$ (red) and auroral intensity (blue) at the highest cross-correlation pixel.

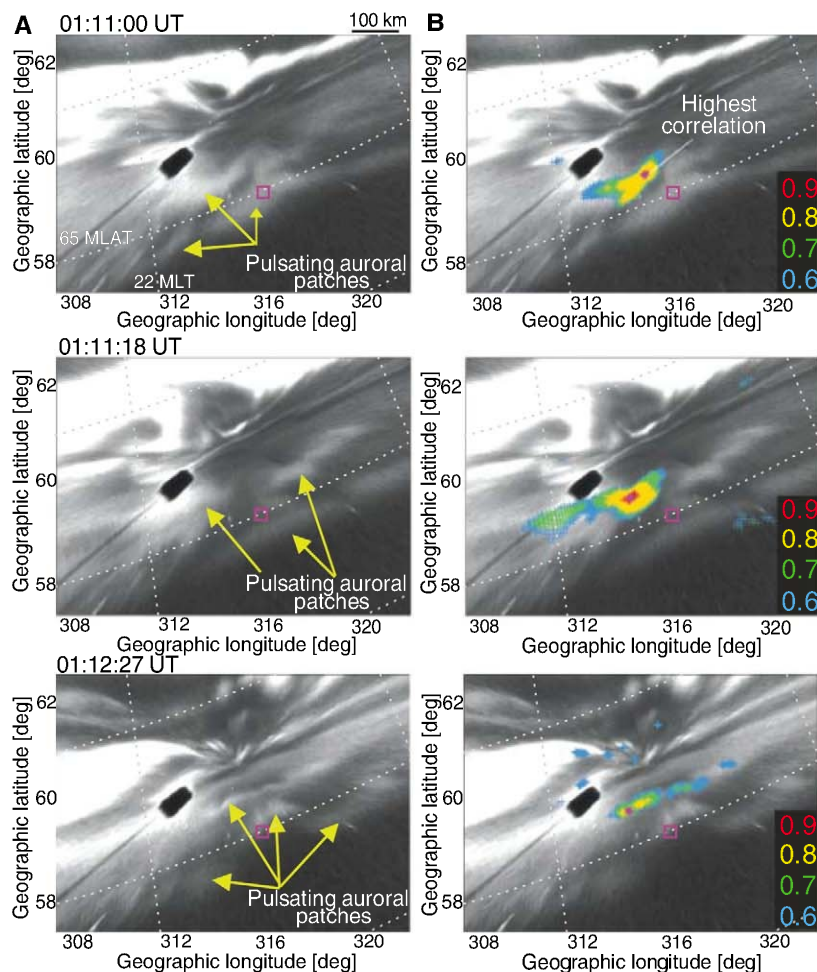


Fig. 2. Spatial distribution of the aurora-chorus cross-correlation during the observations of Fig. 1. (A) PA and (B) cross-correlation coefficient superimposed onto the images during the 1-min time interval, including each snapshot time in (A). Pixels with correlation coefficient below 0.6 are not color-coded. The image format is the same as in Fig. 1C.

auroral patch that is correlated with the chorus intensity variation. The highest cross-correlation (0.91) is located 0.83° MLAT north and 0.07 hour in magnetic local time (MLT) (80 km) west of the model footprint.

The cross-correlation coefficients calculated for the later images (second and third rows in Fig. 2B) are spatially grouped on a pulsating patch as in the top row, again indicating that only a single pulsating patch is correlated with the chorus intensity. While the patch shape changes with time, the location of highest correlation stays essentially fixed, providing further support for the association of the PA to wave-induced precipitation originating at the spacecraft location.

The dominant role of lower-band chorus in driving the PA is further illustrated in Fig. 3 in different times (24). During the longer-duration chorus event (Fig. 3A and movie S2), the time series of the wave intensity and auroral luminosity were again in close agreement (the correlation coefficient is 0.71), indicating that the duration of each occurrence of PA is controlled by the modulation of lower-band chorus (25).

For the event shown in Fig. 3B measured in another time period, lower-band chorus was negligible but intense ECH waves were present. As shown in movie S1, diffuse aurora without pulsations extended over a wide latitudinal range near the longitude of the spacecraft after 01:15 UT. The cross-correlation analysis found the diffuse aurora region as having the highest correlation (0.73) with the ECH waves. A comparison of the time series (Fig. 3B, bottom panel) captures the simultaneous auroral intensification and the pronounced enhancement of ECH intensity. The aurora, however, did not pulsate but stayed at a high intensity after the initial increase, indicating that ECH waves can indeed (11) contribute

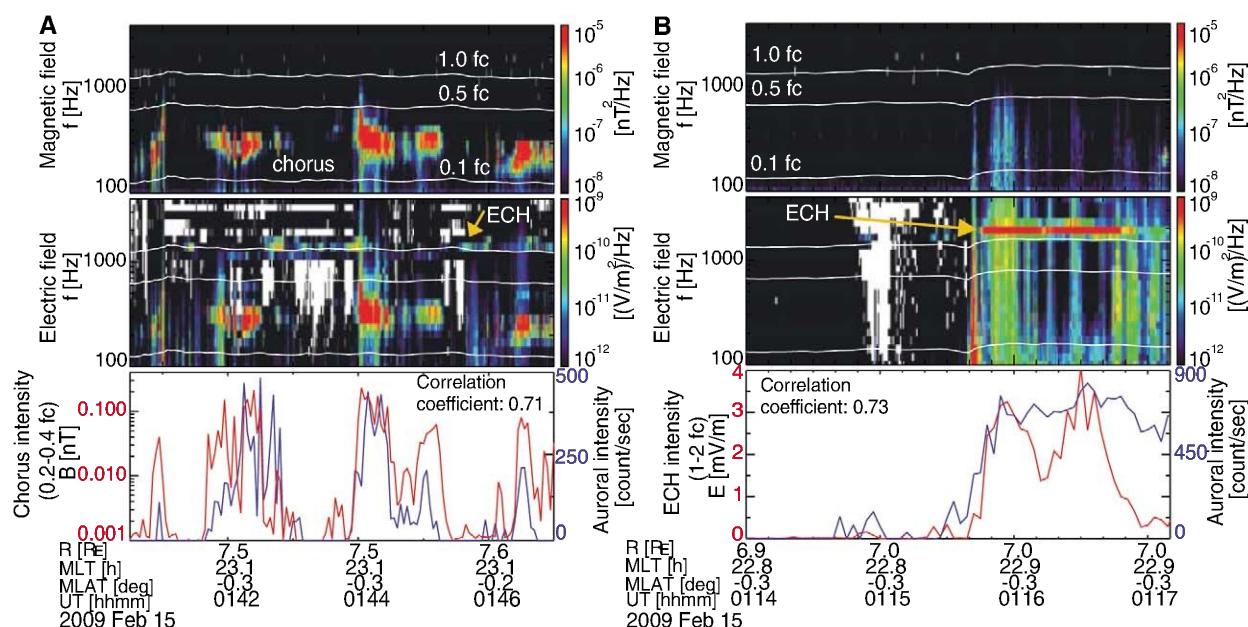


Fig. 3. THEMIS-A-imager correlation analysis results during (A) longer-duration chorus and (B) ECH events. The format is the same as in Fig. 1, B and D. The entire auroral sequences are given in movie S2 and the latter part of movie S1.

to the diffuse aurora but are not responsible for the pulsating patches (26).

Theoretical calculations (9, 27) using the observed lower-band chorus amplitudes (~50 pT) show that such amplitudes are sufficiently strong to cause precipitation of ~10-keV electrons into the atmosphere. Wave burst observations with higher resolution (fig. S1) indicate more intense fields (~30 mV/m and ~1 nT) that were quasi-field-aligned and propagated away from the equator, making the interaction with electrons that precipitate into the atmosphere and drive PA substantially more efficient near the equator (less than ~15°) because of Landau damping and increasing resonant energy in latitude. Each chorus element generated near the equator with a characteristic size of ~100 to 3000 km (7, 28) and the oblique propagation away from the original magnetic field line (10, 29) would lead to interaction regions with a typical patch size (~100 km in the ionosphere and ~a few thousand km in the magnetosphere).

Magnetic field line mapping has been a problematic issue in magnetosphere-ionosphere coupling studies. Based on the property that the high-correlation region occurs over a single auroral patch with the correlation coefficient outside this area diminishing quickly with distance, PA can be used to highlight the physical link between chorus wave activity and each auroral pul-

sation. The PA thus provides a unique opportunity to identify the footprint of the magnetic field line threading the spacecraft, to a precision within the auroral patch size (less than ~100 km) and possibly even down to a few imager pixels (~km).

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24. We also analyzed other events (table S1).
25. Weak ECH waves that may lead to weak auroral emission were also observed in the electric field spectra above 1.0 f_c .
26. The auroral intensity did not diminish following the decrease in the ECH intensity but stayed at a high level. This is attributable to the long lifetime (~1 min) of excited oxygen atom at higher altitudes (32) due to the precipitation of lower-energy electrons (~1 keV), as expected from resonance with ECH (11).
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33. This work was supported by NASA contract NAS-052099, 9F007-046101; NSF grants ATM-0802843 and AGS-0840178; and a Research Fellowship from the Japan Society for the Promotion of Science. The French involvement (Search-coil magnetometer) on THEMIS is supported by CNES and CNRS.

Supporting Online Material

www.sciencemag.org/cgi/content/full/330/6000/81/DC1

Fig. S1

Table S1

Movies S1 and S2

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Cellodextrin Transport in Yeast for Improved Biofuel Production

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Fungal degradation of plant biomass may provide insights for improving cellulosic biofuel production. We show that the model cellulolytic fungus *Neurospora crassa* relies on a high-affinity cellodextrin transport system for rapid growth on cellulose. Reconstitution of the *N. crassa* cellodextrin transport system in *Saccharomyces cerevisiae* promotes efficient growth of this yeast on cellodextrins. In simultaneous saccharification and fermentation experiments, the engineered yeast strains more rapidly convert cellulose to ethanol when compared with yeast lacking this system.

The bioethanol industry uses the yeast *Saccharomyces cerevisiae* to ferment sugars derived from cornstarch or sugarcane into ethanol (1). Plant cell walls provide an abundant alternate sugar source yet remain largely unused (2, 3). Present strategies for using plant biomass use large quantities of cellulase cocktails to release

glucose from plant cell walls, posing economic and logistical challenges for implementation of these processes (4). Furthermore, *S. cerevisiae* cannot ferment the cellodextrins naturally released by cellulases (5, 6) and require cellulase cocktails supplemented by β -glucosidases to quantitatively produce fermentable glucose (7).

In contrast to *S. cerevisiae*, cellulolytic fungi grow well on cellodextrins. The model cellulolytic fungus *Neurospora crassa*, when grown on pure cellulose, increases transcription of seven major facilitator superfamily (MFS) sugar transporters as well as an intracellular β -glucosidase (8). A strain carrying a deletion for one of these predicted transporters, NCU08114, grew slowly on cellulose (fig. S1) (9), and strains lacking either NCU08114 or NCU00801 poorly consumed cellobiose, cellotriose, and cellotetraose (fig. S2).

Orthologs of NCU08114 and NCU00801 are widely distributed in the fungal kingdom (Fig. 1A) (10–13), and recent whole-genome transcriptional profiling studies show their importance to interactions between fungi and plants. For example, some cellulolytic fungi increase expression levels of NCU08114 orthologs while degrading plant wall material (10), and the Périgord black truffle increases the expression of a NCU00801 ortholog during symbiotic interactions with plant roots (11). In addition, certain yeasts that grow on cellobiose contain orthologs of NCU08114 and NCU00801 (12). All of these organisms also contain genes for intracellular β -glucosidases (10), suggesting that cellodextrin transport systems are widespread in nature and are essential for optimal growth of fungi on cellulose-derived sugars.

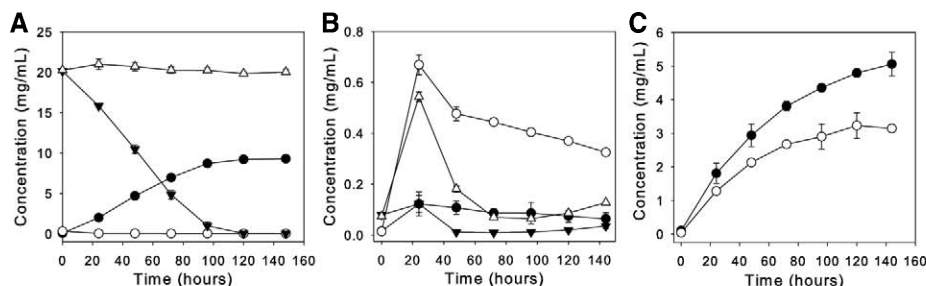
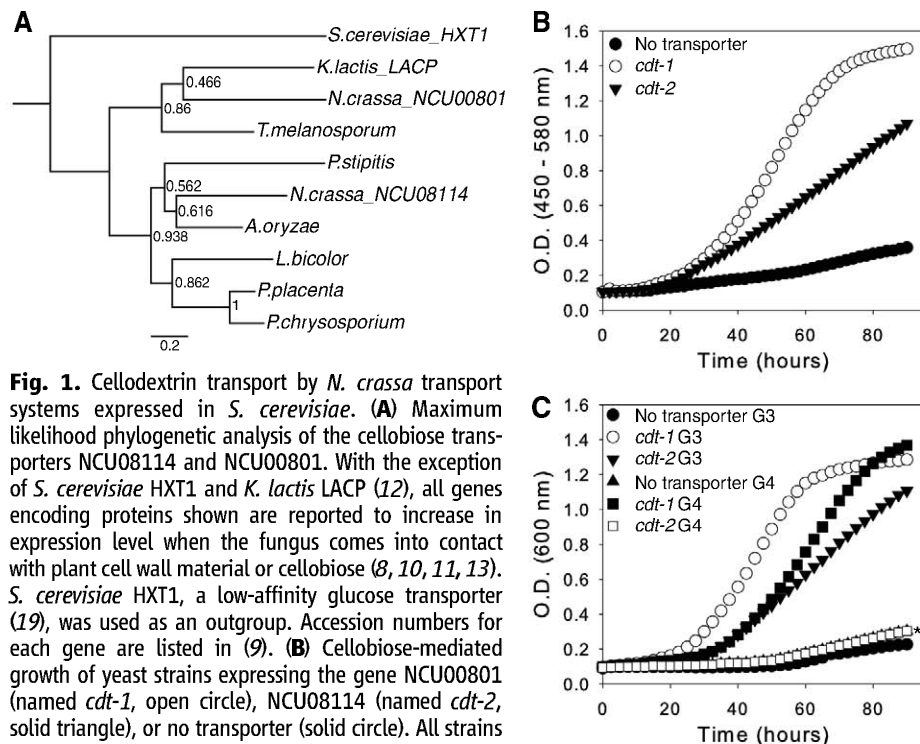
Because cellobiose is not catabolized by *S. cerevisiae* (5, 6) and is not accumulated in the cytoplasm (fig. S3), we reasoned that expression of a functional cellodextrin transport system from *N. crassa* might allow *S. cerevisiae* to grow with cellobiose as the sole carbon source. Yeast strains expressing NCU00801 or NCU08114, together with the intracellular β -glucosidase NCU00130 (hereafter named GH1-1), grew on cellobiose at rates of about 30 and 12% of the rate of *S. cerevisiae* on glucose, respectively (Fig. 1B and fig. S4). Cellodextrins longer than cellobiose also support the growth of yeast expressing these transporters (Fig. 1C), indicating that cellodextrins longer than cellobiose are transported by NCU00801 and NCU08114, hereafter called CDT-1 and CDT-2,

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respectively. Growth cannot be explained by the extracellular hydrolysis of cellodextrins to glucose followed by transport because a strain expressing only the intracellular β -glucosidase GH1-1 grew slowly on cellodextrins (Fig. 1, B and C, and fig. S4). Fur-

thermore, the activity of β -glucosidase GH1-1—which is able to hydrolyze cellobiose, cellotriose, and cellotetraose (fig. S5)—is negligible in culture supernatants and cannot explain the growth of yeast expressing the transporters (fig. S6).



To directly assay transporter function, the uptake of [³H]-cellobiose into yeast cells was measured. Both CDT-1 and CDT-2 are high-affinity cellobiose transporters, with Michaelis constant (K_m) values of 4.0 ± 0.3 μ M and 3.2 ± 0.2 μ M (mean $\pm$ SD), respectively (fig. S7). The expression-normalized maximum velocity (V_{max}) of CDT-1 is over twice that of CDT-2 (fig. S7), which might explain differences in the observed growth rates of the yeast strains (Fig. 1, B and C). Cellobiose transport by CDT-1 and CDT-2 is inhibited by excess cellobiose, and CDT-1 activity is also inhibited by cellotetraose (fig. S8). These data further support that CDT-1 and CDT-2 directly transport the longer cellodextrins in growth assays (Fig. 1C). Thus, the combinations of CDT-1 or CDT-2 with GH1-1 constitute fully functional cellodextrin transport systems.

We next tested whether, in addition to growth, a complete cellodextrin catabolism pathway would be useful in *S. cerevisiae* for lignocellulosic bio-fuel production. Fungal cellulases evolved to function in conjunction with the consumption of sugars released from plant cell walls and work most effectively in this context (14). Simultaneous saccharification and fermentation (SSF) mimics this natural synergy by the addition of fermenting microbes to the plant biomass depolymerization reaction, resulting in a more efficient process (15, 16). Current SSF schemes are limited by the need for quantitative conversion of cellulose to glucose outside yeast cells (7). A cellodextrin transport system could be particularly useful during SSF because it would remove the requirement for full hydrolysis of cellulose to glucose by extracellular β -glucosidases (17) and would reduce the risk of contamination by glucose-dependent organisms (18). Furthermore, both CDT-1 and CDT-2 have a higher apparent affinity for cellobiose ($K_m \approx 3$ to 4 μ M) (fig. S7) when compared with secreted fungal β -glucosidases

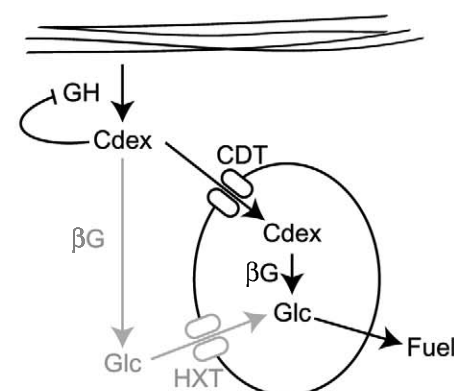


Fig. 3. Use of cellodextrin transport pathways from filamentous fungi in yeast during simultaneous saccharification and fermentation of cellulose. The cellodextrin (Cdex) transport pathway (black) includes a cellodextrin transporter (CDT) and intracellular β -glucosidase (β G). The sugar catabolism pathway present in standard yeast includes hexose transporters (HXT). In SSF, cellulases (GH) and extracellular β -glucosidase (β G) may both be used.

[$K_m \approx 100$ to $1000 \mu\text{M}$ (17)] and to *S. cerevisiae* hexose transporters' apparent affinity for glucose [$K_m \approx 1000$ to $10,000 \mu\text{M}$ (19)]. Therefore, the celldextrin transport systems should more effectively maintain soluble sugar levels below the concentration at which they inhibit fungal cellulases [inhibition constant (K_i) of cellobiose ≈ 19 to $410 \mu\text{M}$ (20)].

With little optimization, yeast expressing *cdt-1* and *ghl-1* fermented cellobiose with an ethanol yield of 0.441 ± 0.001 (grams of ethanol/grams of glucose $\pm$ SD), which is 86.3% of the theoretical value (Fig. 2A) (21). This yield is close to present industrial yields of ethanol from glucose of 90 to 93% (22). Yeasts expressing a celldextrin transport system markedly improve the efficiency of SSF reactions by reducing the steady-state concentration of both cellobiose and glucose and by increasing the ethanol production rate (Fig. 2, B and C). The addition of a celldextrin transport system to biofuel-producing strains of yeast (Fig. 3) overcomes a major bottleneck to fermentation of lignocellulosic feedstocks and probably will help to make cellulosic biofuels economically viable.

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Supporting Online Material

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Figs. S1 to S8

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Sequencing of *Culex quinquefasciatus* Establishes a Platform for Mosquito Comparative Genomics

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Culex quinquefasciatus (the southern house mosquito) is an important mosquito vector of viruses such as West Nile virus and St. Louis encephalitis virus, as well as of nematodes that cause lymphatic filariasis. *C. quinquefasciatus* is one species within the *Culex pipiens* species complex and can be found throughout tropical and temperate climates of the world. The ability of *C. quinquefasciatus* to take blood meals from birds, livestock, and humans contributes to its ability to vector pathogens between species. Here, we describe the genomic sequence of *C. quinquefasciatus*: Its repertoire of 18,883 protein-coding genes is 22% larger than that of *Aedes aegypti* and 52% larger than that of *Anopheles gambiae* with multiple gene-family expansions, including olfactory and gustatory receptors, salivary gland genes, and genes associated with xenobiotic detoxification.

Mosquitoes are the most important vectors of human disease and are responsible for the transmission of pathogens

that cause malaria (*Anopheles*), yellow fever and dengue (*Aedes*), as well as lymphatic filariasis and encephalitis viruses (*Culex*, *Aedes*, *Anopheles*).

Sequencing the *Anopheles gambiae* and *Aedes aegypti* genomes has provided important insights into the genomic diversity underlying the complexity of mosquito biology (1, 2). We describe the sequencing of the *Culex quinquefasciatus* (the southern house mosquito) genome, which offers a reference genome from the third major taxonomic group of disease-vector mosquitoes. With more than 1200 described species, *Culex* is the most diverse and geographically widespread of these three mosquito genera. Apart from contributing to the spread of West Nile encephalitis, it also transmits St. Louis encephalitis and other viral diseases and is a major vector of the parasitic *Wuchereria bancrofti* nematode that has caused the majority of the 120 million current cases of lymphatic filariasis (3).

Taxonomy of the *Culex pipiens* species complex is the subject of a long-standing debate, an issue complicated by the occurrence of viable species hybrids in many geographic areas [reviewed in (4, 5)]. We followed the standard set by the National Center for Biotechnology Information and refer to the species sequenced here as *C. quinquefasciatus*. The Johannesburg strain of *C. quinquefasciatus* was established from a single pond in Johannesburg, South Africa—an area where the two taxa, *C. quinquefasciatus* and *C. pipiens*, were found to be sympatric [(5) therein described as subspecies *C. pipiens quinquefasciatus* and *C. pipiens pipiens*] but have remained much more genetically distinct than the same two sympatric taxa found in California.

We were able to map 9% of the *C. quinquefasciatus* genes (1768 genes) on the three chromosomes with the use of published and new *C. quinquefasciatus* and *Ae. aegypti* markers (6). Of these mapped genes, 803 had *An. gambiae* orthologs and 641 had *Drosophila melanogaster*

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orthologs, consistent with the established species phylogeny (Fig. 1A). Examining correlations between chromosomal arms indicated whole-chromosome conservation between *C. quinquefasciatus*, *An. gambiae*, and *D. melanogaster* (Fig. 1B) (6), whereas—and as suggested from earlier work (7)—*Ae. aegypti* appears to have experienced an arm exchange between the two longest chromosomes after the *Aedes/Culex* divergence (fig. S1).

A significant fraction of the assembled *C. quinquefasciatus* genome (29%) was composed of transposable elements (TEs) (fig. S2). This amount is less than the TE fraction of *Ae. aegypti* (42 to 47%), but greater than that of *An. gambiae* (11 to 16%) (1, 2, 6), suggesting an increased level of TE activity and/or reduced intensity of selection against TE insertions in the two culicinae lineages since their divergence from the *An. gambiae* lineage. A comparative analysis of the age distribution of the different TE types in the three sequenced mosquito genomes revealed that retrotransposons have consistently been the dominant TE type in the *Ae. aegypti* lineage over time (fig. S3). More recently, retrotransposons have become the predominant type of TEs active in all three species.

The *C. quinquefasciatus* repertoire of 18,883 protein-coding genes is 22% larger than that of *Ae. aegypti* (15,419 genes) and 52% larger than that of *An. gambiae* (12,457 genes) (Fig. 1C). Our estimated gene number combines ab initio and similarity-based predictions from three independent automated pipelines, optimizing gene identification (6). The relative increase in *C. quinquefasciatus* gene number is explained in part by the presence of substantially more expanded gene families, including olfactory and gustatory receptors, immune-related genes, and genes with possible xenobiotic

detoxification functions (table S1). Expert curation of selected gene families revealed expansions in cytosolic glutathione transferases and a substantial expansion of cytochrome P450s. A large cytochrome P450 repertoire may reflect adaptations to polluted larval habitats and may have played a role in rendering this species particularly adaptable to evasion of insecticide-based control programs, with several *C. quinquefasciatus* P450s being associated with resistance (8, 9).

Mosquitoes are the subject of intense efforts aimed at designing novel vector control methods that are often based on the ability of the insect to sense its environment (10, 11). *C. quinquefasciatus* has the largest number of olfactory-receptor-related genes (180) of all dipteran species examined to date (table S1). This expansion may reflect culicine olfactory behavioral diversity, with particular regard to host and oviposition site choice. *C. quinquefasciatus* females are opportunistic feeders, being able to detect and feed on birds, humans, and livestock, depending on their availability. This plasticity in feeding behavior contributes to the ability of *C. quinquefasciatus* to vector pathogens, such as West Nile virus and St. Louis encephalitis virus, from birds to humans. The repertoire of gustatory receptors, which are known to mediate perception of both odorants and tastants (12), has also expanded in *C. quinquefasciatus*, primarily through a large alternatively spliced gene locus.

The saliva of blood-sucking arthropods contains a complex cocktail of pharmacologically active components that disarm host hemostasis (13). The ability of *C. quinquefasciatus* to feed on birds, humans, and livestock would suggest that it contains an expanded number of proteins that would increase its ability to imbibe blood from multiple host species. Consistent with this idea, a large protein family

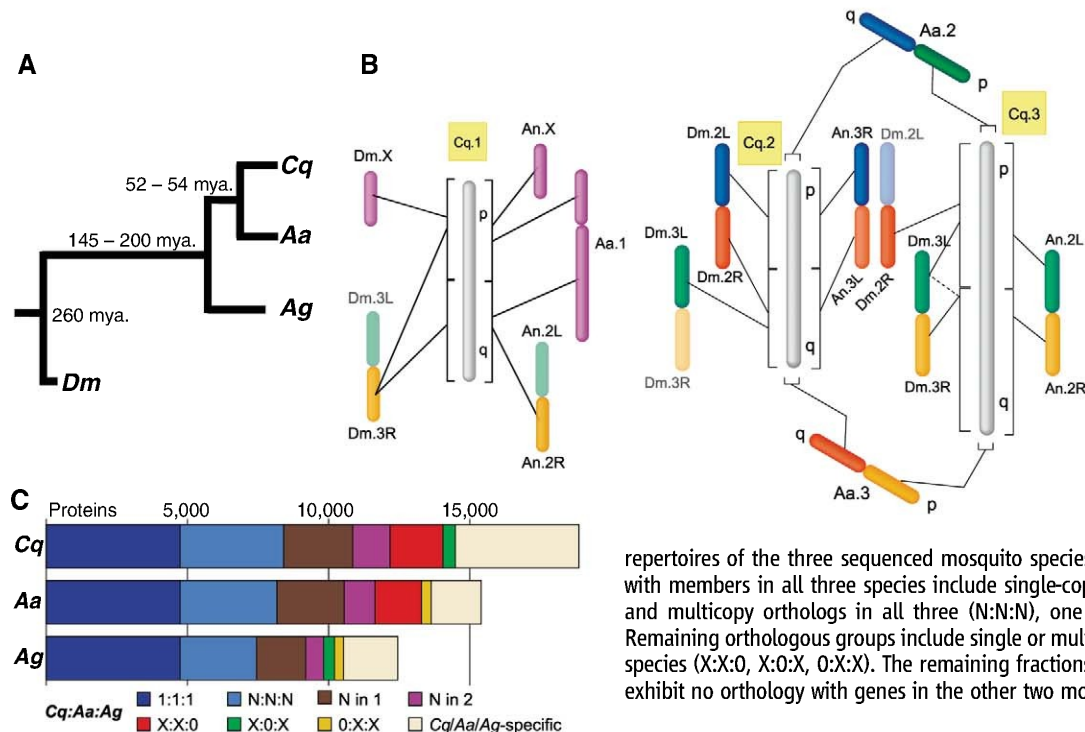


Fig. 1. (A) Codon-based estimates of DNA substitutions along the mosquito phylogeny: *C. quinquefasciatus* (Cq), *Ae. aegypti* (Aa), and *An. gambiae* (Ag) with *D. melanogaster* (Dm) as an outgroup. Dates of divergence were taken from previous studies (6). mya, million years ago. **(B)** Chromosomal synteny between *C. quinquefasciatus*, *Ae. aegypti*, *An. gambiae*, and *D. melanogaster*. Solid lines indicate main orthologous chromosomes; the dashed line denotes secondary orthologous chromosomes. Colors indicate syntenic chromosome arms. Chromosomes are not drawn to scale. **(C)** Orthology delineation among the protein-coding gene

repertoires of the three sequenced mosquito species. Categories of orthologous groups with members in all three species include single-copy orthologs in each species (1:1:1) and multicopy orthologs in all three (N:N:N), one (N in 1), or two (N in 2) species. Remaining orthologous groups include single or multicopy groups with genes in only two species (X:X:0, X:0:X, 0:X:X). The remaining fractions in each species (Cq/Aa/Ag-specific) exhibit no orthology with genes in the other two mosquitoes.

unique to the *Culex* genus, the 16.7 kD family, was previously discovered after salivary transcriptome analysis (13). The genome of *C. quinquefasciatus* revealed 28 additional members of this family.

We have outlined and quantified general similarity and differences at the chromosomal and genomic levels between three disease-vector mosquito genomes, building a foundation for more in-depth future analyses. We found substantial differences in the relative abundance of TE classes among the three mosquitoes with sequenced genomes. Most unexpectedly, this study revealed numerous instances of expansion of *C. quinquefasciatus* gene families compared with *An. gambiae* and the more closely related *Ae. aegypti*. The consequent diversity in many different genes may be an important factor that led to the wide geographic distribution of *C. quinquefasciatus*.

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14. This work was supported by NIH grant HHSN266200400039C and by the National Institute of Allergy and Infectious Diseases, NIH, Department of Health and Human Services under contract numbers N01-AI-30071 and HHSN266200400001C. The assembled genome was deposited in the GenBank database with accession number AAWU000000000.

Supporting Online Material

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Pathogenomics of *Culex quinquefasciatus* and Meta-Analysis of Infection Responses to Diverse Pathogens

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The mosquito *Culex quinquefasciatus* poses a substantial threat to human and veterinary health as a primary vector of West Nile virus (WNV), the filarial worm *Wuchereria bancrofti*, and an avian malaria parasite. Comparative phylogenomics revealed an expanded canonical *C. quinquefasciatus* immune gene repertoire compared with those of *Aedes aegypti* and *Anopheles gambiae*. Transcriptomic analysis of *C. quinquefasciatus* genes responsive to WNV, *W. bancrofti*, and non-native bacteria facilitated an unprecedented meta-analysis of 25 vector-pathogen interactions involving arboviruses, filarial worms, bacteria, and malaria parasites, revealing common and distinct responses to these pathogen types in three mosquito genera. Our findings provide support for the hypothesis that mosquito-borne pathogens have evolved to evade innate immune responses in three vector mosquito species of major medical importance.

The Southern house mosquito, *Culex quinquefasciatus*, is a geographically widespread, often abundant mosquito that is an epidemiologically important vector for an exceptionally diverse array of pathogens, including multiple arboviruses, filarial worms, and protozoa. *C. quinquefasciatus* transmits West Nile virus (WNV), St. Louis encephalitis virus, and other arboviruses, and acts as the most important vector of the causative agent of lymphatic filariasis, *Wuchereria bancrofti*, and *Plasmodium relictum*, an avian malaria parasite. Despite the public health importance of *C. quinquefasciatus*, knowledge of the insect's response capacities to this diverse array of pathogens is limited.

Availability of the *C. quinquefasciatus* genome sequence (1) enabled comparative phylogenomic analyses with *Aedes aegypti* (2), *Anopheles gambiae*

(3), and *Drosophila melanogaster* (4) that identified 500 *C. quinquefasciatus* immunity genes from 39 (sub)families or processes (table S1). Conservation of *C. quinquefasciatus* gene family members follows the species phylogeny, showing greatest similarities with *A. aegypti*. Expansions of C-type lectins (CTLs), fibrinogen-related proteins (FREPs), and serine protease inhibitors (SRPNs) account for much of the 20 to 30% increase in *C. quinquefasciatus* immunity gene number compared with *A. aegypti* (417 genes) and *A. gambiae* (380 genes) (figs. S1 to S4). This apparent diversification in immune surveillance and immune signal amplification processes seems consistent with selection driven by polluted, microbially complex habitats in which *C. quinquefasciatus* oviposits and develops (5).

Whole genome microarray analysis revealed dynamic changes in infection response gene

(IRG) transcription in WNV-infected mosquitoes (fig. S5). Significant changes are observed for 22 transcripts in the midgut and 309 in the carcass (i.e., the remainder of the body) at 7 days postinfection (dpi), with the greater number of IRGs in the latter apparently reflecting the diversity of infected cell and tissue types in the carcass. At 14 dpi, more IRGs are modulated in midgut (539) and carcass (490) when WNV infection has spread in midgut cells and has disseminated to the salivary glands (6). Few canonical immunity genes are represented among *C. quinquefasciatus* WNV IRGs (fig. S5). Five CTL genes within a *C. quinquefasciatus*-specific gene expansion (fig. S3) are up-regulated. Several genes related to the

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Toll, Imd, and JAK-STAT pathways, including *Spätzle*, *REL1*, *IAP2*, and *STAT* orthologs, are activated in *C. quinquefasciatus* and in *A. aegypti* (7, 8) by WNV and dengue virus (DENV) infection, respectively, further supporting a key role of these defense systems in controlling viral pathogens.

Although the *C. quinquefasciatus* genome encodes orthologs for all components of the antiviral defense RNA interference (RNAi) pathway (9), none of them is transcriptionally modulated significantly during WNV infection. Similarly, RNAi components are not transcriptionally modulated during arbovirus infection in *A. aegypti* (10), even though RNAi function is key to limiting these infections in mosquitoes (10–12). Apoptosis is evident, and *C. quinquefasciatus* *IAP1* is repressed in WNV-infected salivary glands (6, 13). However, no significant changes in transcript abundance for caspases, caspase activators, *IAP* genes (other than *IAP2*), or autophagy-related genes are evident in WNV-infected *C. quinquefasciatus*, even though modulation of apoptosis (14) or autophagy (15) pathway function affects viral infection in flies. The nonresponsiveness of these genes appears to reflect the persistent and generally noncytolytic nature of arbovirus infections in a susceptible vector; overt activation of these responses would counteract virus persistence and transmission.

Comparative analysis of expressed sequence tags (tables S3 and S4) from *W. bancrofti*-infected *C. quinquefasciatus* revealed many novel IRGs, presumably because infection with a large metazoan parasite inflicts traumatic injury. Infection with non-native bacteria elicits acute cellular and

humoral immune responses in *C. quinquefasciatus* and other vector mosquitoes (16–18). About 60% of *W. bancrofti* or bacteria IRGs are of diverse or unknown function (fig. S6), and only small proportions (4% *W. bancrofti* and 6% bacteria) are immunity genes. Comparison of *C. quinquefasciatus* virus, filarial worm, and bacteria IRGs reveals unexpected and extensive overlap (548 genes) between *W. bancrofti* and bacteria IRGs (Fig. 1A and fig. S6). Overall, 38 *C. quinquefasciatus* IRGs are common among all three infections (table S5).

The identification of *C. quinquefasciatus* IRGs provided an unprecedented opportunity to undertake a meta-analysis of 25 vector-pathogen interactions in *C. quinquefasciatus*, *A. aegypti*, and *A. gambiae* infected with arboviruses, parasites, and bacteria (Fig. 1B and table S6) within the context of orthologous groups (OGs) that define evolutionarily related genes. A set of 69 arbovirus IRG OGs (representing 93 *C. quinquefasciatus* and 89 *A. aegypti* genes) was implicated in *C. quinquefasciatus*-WNV, *A. aegypti*-DENV, and *A. aegypti*-Sindbis virus (SINV) responses (fig. S7 and table S7). A cytochrome P450 DENV IRG from *Drosophila* (*Cyp6a19*, FBgn0033979) and mammalian cells (19) is similar to genes that respond significantly in *C. quinquefasciatus*-WNV (CPIJ004411) infection and in *A. aegypti*-SINV and *A. aegypti*-DENV (AAEL009117) infections, highlighting the potential importance of this molecule as a universal arbovirus IRG. Filarial worm IRGs comprised 41 OGs modulated during *C. quinquefasciatus*-*W. bancrofti* infection and infection of *A. aegypti* with *Brugia malayi* (fig.

S8 and table S8). The IRGs represented most frequently include serine proteases and cuticle proteins. Changes in the latter may be associated with tissue repair necessitated by parasite invasion, migration, and development (20). Increased representation of heat shock protein and cytochrome P450 IRGs appears to reflect stress during the infection response. The most extensive overlap (113 OGs) in bacterial IRGs was observed between Culicine mosquitoes, *C. quinquefasciatus*, and *A. aegypti* (table S9). Only 34 OGs and 26 OGs represent IRGs (fig. S9) in bacteria-infected *C. quinquefasciatus* and *A. gambiae* (table S10) and *A. aegypti* and *A. gambiae*, respectively. Among 16 OGs containing bacteria IRGs from all three species, serine proteases, cecropins, myosin light chain, and components of the 26S proteasome are highly represented (table S11). A meta-analysis of bacteria, filarial worm, virus, and malaria parasite infection data sets from *C. quinquefasciatus*, *A. aegypti*, and *A. gambiae* reveals 95 orthologous IRGs that span mosquito species and pathogen types (Fig. 1B, fig. S10, and table S12).

Orthology data (21) were employed to distinguish universal (see Fig. 1) multi- or single-copy OGs from mosquito-specific OGs, revealing that the majority of IRGs have orthologs across Arthropoda (Fig. 1C and table S13). Universal multicopy OGs are overrepresented among IRGs for viruses, filaria, and bacteria; and universal single-copy OGs are underrepresented among arbovirus and filarial worm IRGs (and among IRGs common to all pathogens in *C. quinquefasciatus*, *A. aegypti*, and *A. gambiae*) compared with the complete set of mosquito OGs (Fig. 1C). Immunity genes (IMM) (Fig. 1D), including CTLs, CLIPs, and SRPNs, are generally more prevalent among responsive multicopy OGs than among responsive single-copy OGs. In fact, no canonical immunity genes are found among arbovirus- or filarial worm-responsive universal single-copy OGs.

The cosmopolitan distribution of *C. quinquefasciatus* across continents and ecozones generally south of 39°N latitude implies that this species has the plasticity to adapt to diverse habitats, and this plasticity may be enhanced by an expanded immunity gene repertoire. Overrepresentation of universal multicopy OGs among pathogen IRGs implies that members of expanded gene families have been recruited into pathogen-responsive defense pathways. Arboviral and filarial worm infections constitute susceptible, long-term vector-pathogen interactions in which the pathogen undergoes amplification or develops intracellularly, whereas acute infections with non-native bacteria trigger systemic immunity and are cleared rapidly (22, 23). Our meta-analysis reveals that arboviral and filarial worm pathogens transmitted by vector mosquitoes modulate very few canonical immunity genes and fail to affect expression of RNAi and most programmed cell death pathway genes in these vectors. Our results therefore provide strong support for the hypothesis that pathogens that successfully develop in, and are transmitted by, vector mosquitoes have

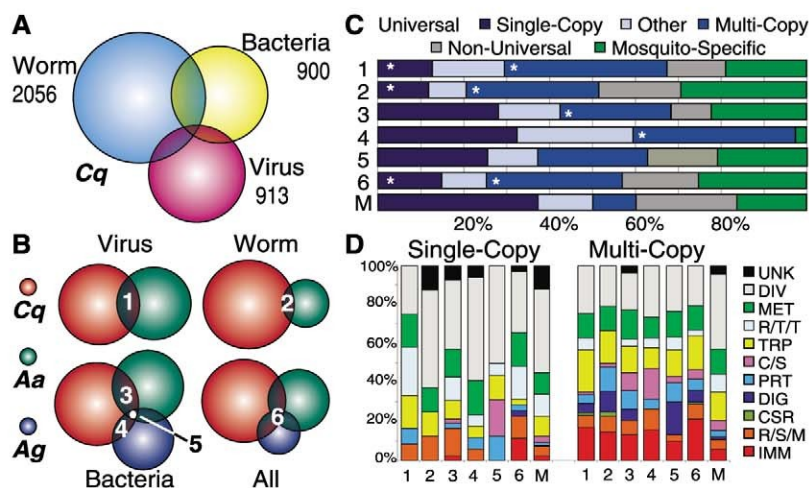


Fig. 1. Infection response genes (IRGs) in the mosquitoes *Culex quinquefasciatus* (Cq), *Aedes aegypti* (Aa), and *Anopheles gambiae* (Ag). **(A)** Shared and unique infection response genes in *C. quinquefasciatus* infected with a filarial worm, bacteria, or virus. **(B)** Proportions of shared and unique IRGs postinfection with viruses (1), filaria (2), or bacteria (3) in *C. quinquefasciatus* and *A. aegypti*, in *C. quinquefasciatus* and *A. gambiae* (4), and in all three species (5); and common IRGs in *C. quinquefasciatus*, *A. aegypti*, and *A. gambiae* (6). **(C)** Orthology relationships for IRG sets (Rows 1 to 6). IRGs with orthologs in at least 20 arthropod species were classified as Universal, as compared to Non-Universal or Mosquito-Specific. Gene copy-number counts distinguish mostly single- and multicopy orthologous groups. IRG sets 1 to 6 were compared to 10,083 mosquito OGs (Row M) to identify significantly greater or smaller (asterisks) proportions (Fisher's Exact Tests; $P < 10^{-5}$). **(D)** Consensus functional categories of universal single-copy (left) and multicopy (right) orthologous groups of IRG sets (Rows 1 to 6), and all mosquito groups (Row M). Functional groups are described in supporting online material and (24). Each set of IRGs is described in tables S7 to S12.

evolved to avoid most immune responses in the three mosquito genera responsible for the vast majority of human morbidity and mortality attributable to insect-transmitted pathogens.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/330/6000/88/DC1

Materials and Methods

Figs. S1 to S12

Tables S1 to S13

References

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A Critical Role for LTA₄H in Limiting Chronic Pulmonary Neutrophilic Inflammation

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Leukotriene A₄ hydrolase (LTA₄H) is a proinflammatory enzyme that generates the inflammatory mediator leukotriene B₄ (LTB₄). LTA₄H also possesses aminopeptidase activity with unknown substrate and physiological importance; we identified the neutrophil chemoattractant proline-glycine-proline (PGP) as this physiological substrate. PGP is a biomarker for chronic obstructive pulmonary disease (COPD) and is implicated in neutrophil persistence in the lung. In acute neutrophil-driven inflammation, PGP was degraded by LTA₄H, which facilitated the resolution of inflammation. In contrast, cigarette smoke, a major risk factor for the development of COPD, selectively inhibited LTA₄H aminopeptidase activity, which led to the accumulation of PGP and neutrophils. These studies imply that therapeutic strategies inhibiting LTA₄H to prevent LTB₄ generation may not reduce neutrophil recruitment because of elevated levels of PGP.

Neutrophils are a critical component of the host's defense against microorganisms (1, 2); however, cessation of neutrophil recruitment and clearance by apoptosis is mandatory to restore homeostasis and limit host tissue damage (3, 4). Chronic neutrophilic inflammation is observed in lung diseases such as cystic fibrosis (CF) and chronic obstructive pulmonary disease (COPD), mediating extensive tissue damage and contributing to organ dysfunction (5, 6). A classical chemoattractant for neutrophils is the glutamic acid-leucine-arginine (ELR⁺) motif containing CXC chemokines (7), such as interleukin (IL)-8 (CXCL8) in humans and keratinocyte-derived chemokine (KC) (CXCL1) and macrophage inflammatory protein (MIP)-2 (CXCL2) in mice. Proline-glycine-proline (PGP) is a

tripeptide generated from the breakdown of extracellular matrix collagen and is specifically chemotactic for neutrophils in vitro and in vivo (8, 9). N-terminal acetylation of PGP (AcPGP), which occurs through an unknown mechanism, can enhance its chemotactic potential (10). PGP and AcPGP share sequence homology to key motifs found in the majority of ELR⁺ CXC chemokines and bind to their receptors, CXCR1 and CXCR2 (8, 9). PGP is generated from native collagen by the action of matrix metalloproteinase-8 (MMP-8) and/or MMP-9, followed by a secondary cleavage by prolyl endopeptidase (PE) (9). We have subsequently identified substantial quantities of PGP or AcPGP in clinical samples from patients with chronic lung diseases such as CF, COPD, and bronchiolitis obliterans syndrome (8, 9, 11, 12),

where it functions to promote the maintenance of neutrophilic inflammation at a time of declining classical chemokine levels. Here, we investigate the role of PGP in acute pulmonary neutrophilic inflammation.

Influenza infection of mice elicits an acute neutrophilic inflammation peaking at 24 hours post-infection (fig. S1, A and B) (13), coinciding with peak KC and MIP-2 levels (fig. S1, C and D). MMP-9 protein expression and activity were elevated at 24 hours post-infection and persisted for several days before subsiding to baseline levels (fig. S1, E to G). PE activity (fig. S1H) and protein amounts (fig. S1I) were also rapidly elevated in bronchoalveolar lavage fluid (BALF) after influenza infection, peaking at 24 hours after infection. Thus, the entire enzyme repertoire required for PGP generation was present. Neutrophils were demonstrated to be a prominent source of both MMP-9 and PE (fig. S1, J and K) (13). Despite the presence of MMP-9 and PE, however, no PGP was detectable at any time point that we analyzed (fig. S2A) with the use of a highly sensitive mass spectrometry technique (8).

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The absence of PGP during influenza infection led us to question whether an enzyme was present that degraded PGP. BALF from 24 hours post-influenza infection (maximal MMP-9/PE) exhibited a potent capacity to degrade exogenous PGP, as determined by mass spectrometry (Fig. 1A). Generation of free proline (from PGP degradation) by influenza BALF correlated with the loss of PGP by mass spectrometry (Fig. 1B), with one proline being cleaved per PGP molecule. N-terminal acetylation of PGP protected it from degradation (fig. S2B). BALF from different time points post-influenza infection was subsequently incubated with PGP, and degradation was assessed by mass spectrometry and free-proline generation (Fig. 1C). Naïve BALF had the capacity to degrade PGP, but activity was markedly increased after influenza infection (peaking at 24 to 48 hours) before subsiding to basal activity. Lung epithelial cells and neutrophils were shown to be prominent sources of this PGP-degrading activity (fig. S2, C to E) (13). Thus, both PGP-generating and -degrading enzymes are similarly up-regulated during influenza infection coinciding with neutrophilic inflammation.

The enzyme responsible for degrading PGP was isolated from influenza BALF and demonstrated to be leukotriene A₄ hydrolase (LTA₄H) (fig. S3 and table S1) (13). LTA₄H acts as an epoxide hydrolase to catalyze the conversion of LTA₄ to the proinflammatory mediator leukotriene B₄ (LTB₄) (14, 15). It has also been suggested to have aminopeptidase capacity, but no physiological substrate has been identified (16, 17). We incubated recombinant human LTA₄H with PGP with and without bovine serum albumin (BSA)—because its aminopeptidase activity is reportedly enhanced by albumin (18)—and assessed PGP degradation (Fig. 2, A and B). LTA₄H had a potent capacity to degrade PGP, which was augmented at lower enzyme concentrations by BSA. Recombinant LTA₄H did not degrade AcPGP (fig. S4A). LTA₄H displayed a significantly greater catalytic constant (k_{cat}) for a PGP substrate than for LTA₄, but it also possessed a greater Michaelis constant (K_M). Consequently, the catalytic efficiency (k_{cat}/K_M) of LTA₄H for PGP was $\sim 1 \times 10^5 \text{ s}^{-1} \text{ M}^{-1}$, which

compares favorably with reported specific activities for LTA₄H with LTA₄ (fig. S4, B to D, and table S2) (13, 17). Murine recombinant LTA₄H was expressed and purified (fig. S5, A to C) and demonstrated to possess a comparable capacity to degrade PGP (fig. S5, D to G).

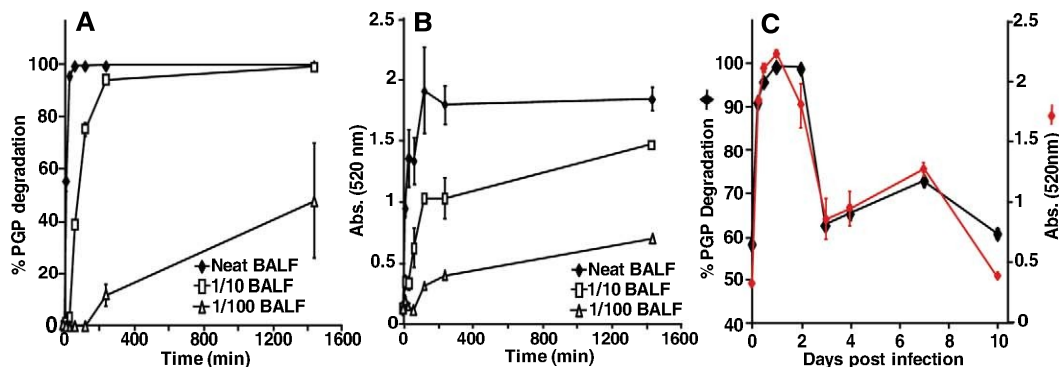
LTA₄H selectively cleaved the N-terminal amino acid from tripeptides and was inhibited by the tripeptide aminopeptidase inhibitor bestatin, but not other general aminopeptidase inhibitors such as amastatin (fig. S6, A and B) (13). Biological samples containing MMP-9/PE can generate PGP from collagen *ex vivo* (9). Although BALF incubated with collagen failed to generate PGP, co-incubation with bestatin yielded small quantities of PGP with naïve BALF and substantial quantities with BALF from 24 hours post-influenza infection (Fig. 2C and fig. S2C). Bestatin or amastatin was subsequently administered intratracheally to influenza-infected mice. Although we did not detect PGP in the BALF from untreated mice at 24 hours post-infection, those administered bestatin generated substantial concentrations of PGP in their BALF (Fig. 2D) and possessed significantly greater numbers of neutrophils in their lung tissue (Fig. 2E). It would, therefore, appear that LTA₄H degrades collagen-derived PGP, limiting neutrophilic inflammation.

To verify a role for LTA₄H in PGP degradation *in vivo*, we next assessed the amount of PGP in the BALF of wild-type (WT) (fig. S7, A to D) (13) and *Lta4h*^{−/−} mice. Naïve WT mice possessed no PGP, whereas *Lta4h*^{−/−} mice exhibited low but reproducible concentrations of PGP (Fig. 3A). This phenotype was more pronounced at day 3 post-influenza infection (peak neutrophil infiltrate) (Fig. 3B). *Lta4h*^{−/−} mice failed to produce LTB₄ but also possessed lower amounts of KC and MIP-2 in BALF relative to wild types (Fig. 3, A and B). There was a small yet significant increase in neutrophil numbers in the lungs of *Lta4h*^{−/−} mice at day 3 of influenza infection (Fig. 3C); however, the contribution of PGP may be partially masked because of the reduced amounts of other chemoattractants. To address this, we measured neutrophil chemotaxis *ex vivo* to WT and *Lta4h*^{−/−} BALF (day 3 post-influenza infection) in

the presence of a PGP-neutralizing antibody. BALF from both groups of mice was chemotactic for neutrophils, and chemotaxis was suppressed by neutralization of KC/MIP-2 (fig. S8A). Neutralization of PGP, however, caused a marked reduction in the chemotactic potential of *Lta4h*^{−/−} BALF only (Fig. 3D). The BALF of WT mice could degrade exogenous PGP, which was markedly increased with influenza infection; however, degradation was absent with BALF from *Lta4h*^{−/−} mice (Fig. 3, E and F). Furthermore, whereas BALF from influenza-infected WT mice generated negligible levels of PGP from collagen *ex vivo* (fig. S8, B and C), BALF from *Lta4h*^{−/−} mice generated significant quantities. PGP generation was significantly reduced by co-incubation with inhibitors to MMP-9 and PE, verifying the importance of these enzymes in the generation of the tripeptide (fig. S8, B and C).

Failure to detect PGP owing to degradation of the tripeptide was not unique to influenza infection, because we obtained comparable results in response to *Streptococcus pneumoniae* (fig. S9) (13). We next questioned why PGP was present in chronic neutrophilic conditions such as COPD (12), because LTA₄H so potently degrades it. Chronic instillation of AcPGP into the lungs of mice results in neutrophil recruitment and an emphysematous lung phenotype (8, 19). We demonstrated that cigarette smoke condensate (CSC) could N-terminally acetylate PGP (Fig. 4A), not only protecting PGP from degradation by LTA₄H but also enhancing its chemotactic capacity (10). Exposure to CSC also dose-dependently inhibited the capacity of LTA₄H to degrade PGP (Fig. 4, B and C). CSC appeared to exhibit selective inhibition of the aminopeptidase activity of LTA₄H, with minimal inhibition of the hydrolase activity observed (Fig. 4D). Furthermore, no difference was observed in LTB₄ generation in response to the chemoattractant formyl-methionine-leucine-phenylalanine when neutrophils were in the presence of CSC (fig. S10). Selective inhibition of aminopeptidase activity is feasible because residues have been delineated that are critical to one activity but have no role in the other (20, 21), and selective modulation has previously been observed

Fig. 1. Influenza infection induces the release of PGP-degrading enzymes. (A and B) Undiluted, diluted by 1/10, or diluted by 1/100 BALF from 24 hours after influenza infection was incubated with PGP, and degradation was assessed by mass spectrometry (A), expressed as percentage degradation relative to peptide alone, or (B) generation of free proline. (C) BALF diluted by 1/10 from indicated times post-influenza infection was incubated with PGP, and degradation was assessed after 2 hours by mass spectrometry or free-proline generation. Data [error bars indicate mean $\pm$ SD (A and B) or mean $\pm$ SEM (C)] are representative of three experiments with triplicates (A and B) or four mice per group (C). Abs, absorbance.



in response to biological and chemical mediators (18, 22, 23). We have previously demonstrated that lung epithelial cells release LTA₄H (fig. S2C). Expo-

sure of primary human bronchial epithelial cells to a nonlethal dose of CSC significantly reduced the aminopeptidase activity of the released LTA₄H (Fig. 4, E and F), while substantially increasing

the detectable amount of total LTA₄H protein released (Fig. 4G). It is important to note that, in this instance, although the apparent percentage inhibition of activity is less than 30%, the amount of LTA₄H protein released by the CSC-stimulated epithelial cells is well in excess of that released by control-treated cells, so the actual inhibition is far greater. The increase in total LTA₄H protein with CSC is in excess of 10-fold; thus, the actual relative inhibition is likely to be in excess of 95%. Furthermore, single exposure of mice to intranasal cigarette smoke extract (CSE) markedly reduced the capacity of BALF (24 hours later) to degrade PGP ex vivo (Fig. 4, H and I) and led to significant concentrations of AcPGP in BALF (Fig. 4J) and neutrophil recruitment (Fig. 4K) in vivo.

In this study, we propose disparate functions of LTA₄H in generating one neutrophil chemoattractant (LTB₄) while degrading another (PGP). LTA₄H is generally assumed to be a cytosolic enzyme, but we report an extracellular role, corroborating detection of this enzyme previously in the plasma of various mammals and in human BALF (24, 25). The peptidase, but not the epoxide hydrolase, activity of LTA₄H is augmented in the presence of albumin (18) and chloride ions (22). The disparate concentrations of Cl⁻ and albumin, intracellularly and extracellularly, support the notion that the epoxide hydrolase activity of LTA₄H operates intracellularly, and the aminopeptidase capacity occurs extracellularly. Neutrophils are a source of enzymes that both generate and degrade PGP and are therefore capable of promoting and then restricting their own recruitment.

Our results also suggest that cigarette smoke shifts the emphasis of LTA₄H, which has dual pro- and anti-inflammatory functions, toward a proinflammatory phenotype. Consequently, a combination of LTB₄ and PGP, both implicated in the

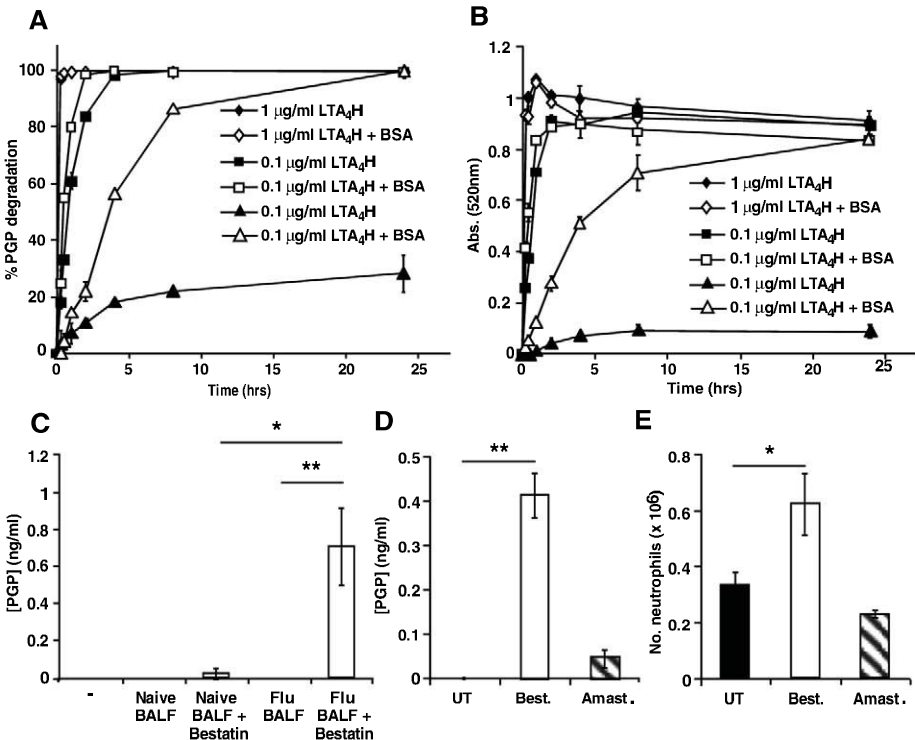
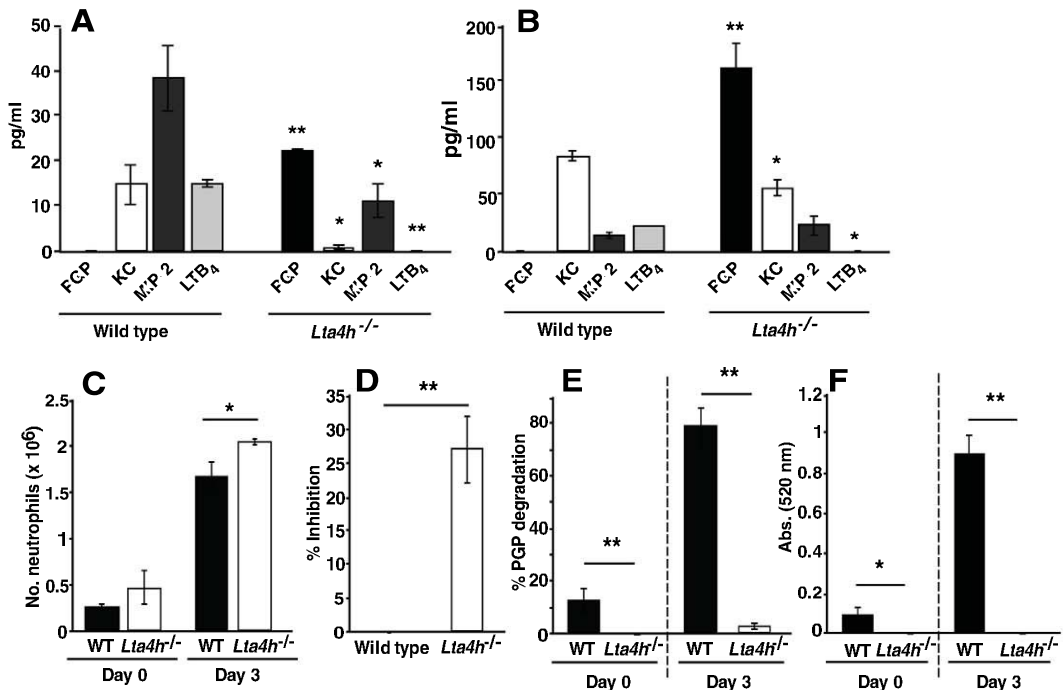


Fig. 2. Leukotriene A₄ hydrolase degrades PGP. (A and B) Degradation of PGP by 1, 0.1, and 0.01 µg/ml recombinant human LTA₄H with or without BSA, as determined by (A) mass spectrometry or (B) generation of free proline. (C) Generation of PGP from type II collagen by BALF from naive and influenza-infected (24 hours) mice, with or without bestatin. (D) PGP in BALF and (E) neutrophil numbers in lung tissue 24 hours after influenza infection in mice that received intratracheal phosphate-buffered saline (untreated), bestatin (Best.), or amastatin (Amast.). **P* < 0.05; ***P* < 0.01. Data [error bars indicate mean ± SD (A) to (C) or mean ± SEM (D) and (E)] are representative of three experiments with triplicates (A) to (C) or four mice per group (D) and (E).

Fig. 3. *Lta4h*^{-/-} mice lose capacity to degrade PGP. The concentrations of KC, MIP-2, LTB₄, and PGP in BALF of (A) naive or (B) influenza-infected (day 3) WT and *Lta4h*^{-/-} mice are shown. (C) Neutrophil numbers in the lungs of naive or influenza-infected (day 3) WT or *Lta4h*^{-/-} mice. (D) Inhibition of WT and *Lta4h*^{-/-} BALF induced neutrophil chemotaxis ex vivo by PGP neutralization. (E and F) BALF, diluted by 1/10, from naive or influenza-infected (day 3) WT and *Lta4h*^{-/-} mice incubated with PGP and degradation assessed after 2 hours by (E) mass spectrometry or (F) free-proline generation. **P* < 0.05; ***P* < 0.01. Data [error bars indicate mean ± SEM (A to C) or mean ± SD (D to F)] are from four mice per group (A) to (C) or triplicates (D) to (F).



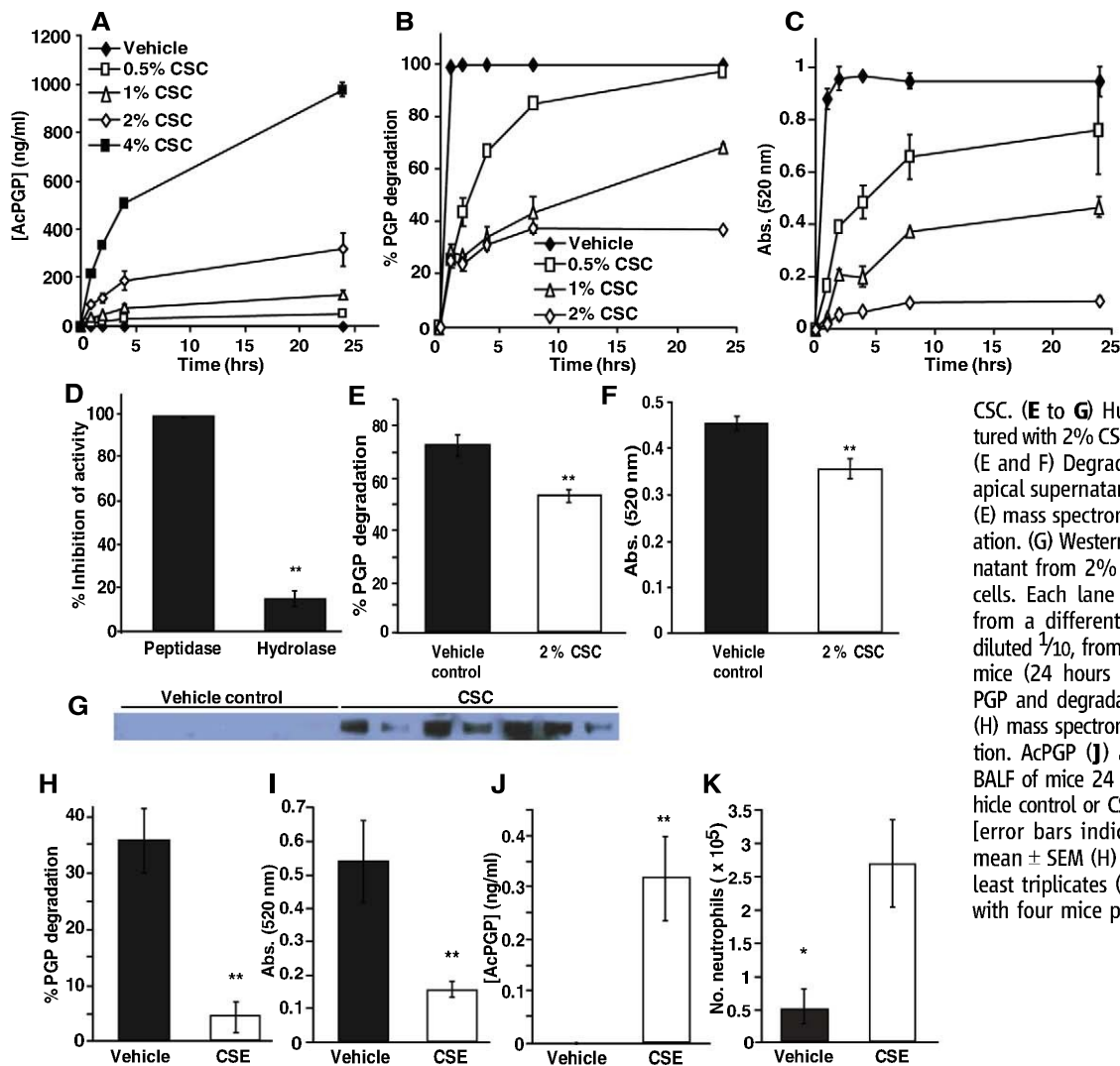


Fig. 4. Cigarette smoke inhibits PGP degradation by LTA₄H. (A) Acetylation of PGP by 0.5, 1, 2, and 4% CSC or vehicle control (2% dimethyl sulfoxide). (B and C) Degradation of PGP by LTA₄H alone or in the presence of 0.5, 1, or 2% CSC or vehicle control, as determined by (B) mass spectrometry or (C) generation of free proline. (D) Inhibition of hydrolase and amino-peptidase activities by 2% CSC. (E to G) Human lung epithelial cells cultured with 2% CSC or vehicle control for 24 hours. (E and F) Degradation of PGP after 2 hours by apical supernatant (diluted 1/10), as assessed by (E) mass spectrometry or (F) free-proline generation. (G) Western blot for LTA₄H in apical supernatant from 2% CSC or vehicle control-treated cells. Each lane represents apical supernatant from a different experiment. (H and I) BALF, diluted 1/10, from vehicle control- or CSE-treated mice (24 hours post-treatment) incubated with PGP and degradation assessed after 2 hours by (H) mass spectrometry or (I) free-proline generation. (J) AcPGP (K) and neutrophil numbers (K) in BALF of mice 24 hours post-administration of vehicle control or CSE. **P* < 0.05; ***P* < 0.01. Data [error bars indicate mean ± SD (A) to (F) or mean ± SEM (H) to (K)] are representative of at least triplicates (A) to (F) or three experiments with four mice per group (H) to (K).

pathogenesis of COPD (8, 12, 19, 26), could drive the observed inflammation. Such selective modulation of the disparate activities of LTA₄H may not be unique to COPD. Extracellular levels of chloride ions, which selectively activate the aminopeptidase activity, are diminished in CF patients owing to defective CF transmembrane conductance regulators (27) and could explain the PGP observed in these patients (9).

Finally, LTB₄ is implicated in acute and chronic inflammatory diseases (28–31). The development of drugs that seek to inhibit LTA₄H activity and LTB₄ generation to alleviate pathologies of inflammatory disease is under way. When developing these drugs, it will be critical to consider the potential repercussions of preventing PGP degradation.

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Materials and Methods
SOM Text

Figs. S1 to S10
Tables S1 and S2

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Mitotic Recombination in Patients with Ichthyosis Causes Reversion of Dominant Mutations in *KRT10*

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Somatic loss of wild-type alleles can produce disease traits such as neoplasia. Conversely, somatic loss of disease-causing mutations can revert phenotypes; however, these events are infrequently observed. Here we show that ichthyosis with confetti, a severe, sporadic skin disease in humans, is associated with thousands of revertant clones of normal skin that arise from loss of heterozygosity on chromosome 17q via mitotic recombination. This allowed us to map and identify disease-causing mutations in the gene encoding keratin 10 (*KRT10*); all result in frameshifts into the same alternative reading frame, producing an arginine-rich C-terminal peptide that redirects keratin 10 from the cytokeratin filament network to the nucleolus. The high frequency of somatic reversion in ichthyosis with confetti suggests that revertant stem cell clones are under strong positive selection and/or that the rate of mitotic recombination is elevated in individuals with this disorder.

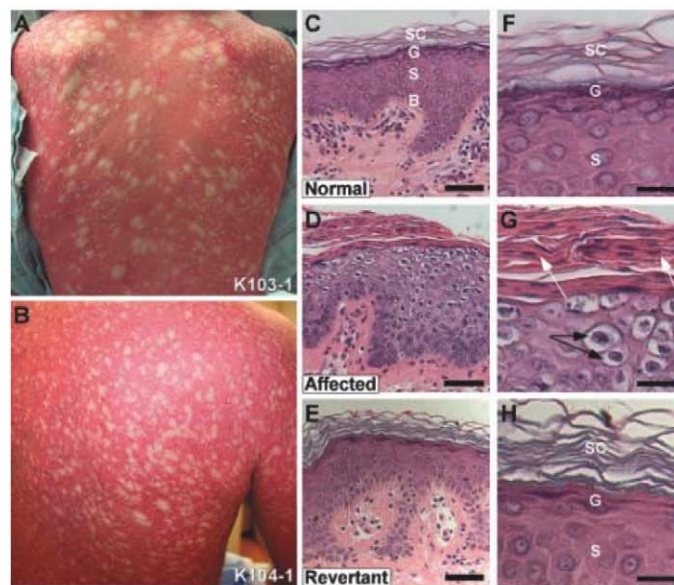
Ichthyosis with confetti (IWC; also known as *ichthyose en cor-fetti*, congenital reticular ichthyosiform erythroderma, and ichthyosis variegata) is a very rare, sporadic severe skin disease of unknown cause (1–3). Affected subjects are born with erythroderma (red skin) owing to defective skin barrier function, prominent scale, and palmoplantar keratoderma (thickening of skin on palms and soles). Poor skin integrity leads to bacterial infections and, frequently, impaired growth and development. Early in life, hundreds to thousands of pale confetti-like spots appear across the body surface and increase in number and size with time (Fig. 1, A and B). Histology of ichthyotic skin shows epidermal thickening and disordered differentiation above the basal layer, with perinuclear vacuolization, lack of a granular layer, and hyperkeratosis (thickening) with retained nuclei in the stratum corneum (Fig. 1, C and D).

We studied seven kindreds with characteristic IWC (fig. S1). In five kindreds, there was a single affected offspring of unaffected, unrelated parents, and in two an affected parent had affected offspring. Biopsy of confetti spots in different

kindreds revealed that these have normal histology (Fig. 1, E to H), consistent with each representing a revertant from clonal expansion of a

normal stem cell. This observation suggested that IWC might be caused by dominant mutations that are lost in revertant spots, and the high frequency of reversion suggested deletion, gene conversion, or recombination as possible mechanisms. To test this, we compared genotypes of DNA from blood and cultured keratinocytes from biopsies of diseased and revertant skin of subject 106-1 typed on Illumina arrays (4). In contrast to blood and disease keratinocytes, revertant DNA showed a single large segment of copy-neutral loss of heterozygosity (LOH) on chromosome 17q extending from 34.5 megabase (Mb) to the telomere at 78.7 Mb (Fig. 2A and fig. S2). Three additional revertant spots from this subject also showed copy-neutral LOH extending from proximal 17q to the telomere, each with different inferred start sites for LOH (Fig. 2B), which excludes simple genetic mosaicism. These findings are consistent with mitotic recombination as the mechanism of LOH (Fig. 2C). In each revertant, the same parental haplotype was lost, consistent with loss of a dominant mutation. We then analyzed 28 revertant spots from five additional patients. Again, all revertants showed copy-neutral LOH on 17q extending to the telomere (Fig. 2B). Sites of inferred recombination are distinct and are

Fig. 1. Frequent revertants in ichthyosis with confetti. (A and B) The backs of an 18-year-old female subject (103-1) and 42-year-old male (104-1) show background redness and scaling with hundreds of white, normal-appearing “confetti” spots. (C) Histology of normal human skin showing basal layer (labeled B), stratum spinosum (S), granular layer (G), and stratum corneum (SC). (D) Affected skin shows loss of differentiation of all layers above the basal layer and hypercellularity with increased epidermal thickness. There is no granular layer, and marked perinuclear vacuolization in the suprabasal epidermis and retention of cell nuclei in the stratum corneum are seen. (E) Revertant skin shows normalization of epidermal thickness and architecture, with normal granular layer, normal spinous layer, and stratum corneum. Scale bars in (C) to (E), 50 μ m. (F) High-power view of spinous layer in normal epidermis with intercellular spines visible, overlying granular layer with purple keratohyalin granules and basket weave stratum corneum. (G) High-power view of affected skin shows perinuclear vacuolization (black arrows), lack of keratohyalin granules, and retained nuclei (white arrows) in the stratum corneum. (H) High-power view of revertant skin shows normal spinous layer with intercellular spines, granular layer with purple keratohyalin granules in keratinocytes, and basket-weave stratum corneum. Scale bars in (F) to (H), 25 μ m.



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There is no granular layer, and marked perinuclear vacuolization in the suprabasal epidermis and retention of cell nuclei in the stratum corneum are seen. (E) Revertant skin shows normalization of epidermal thickness and architecture, with normal granular layer, normal spinous layer, and stratum corneum. Scale bars in (C) to (E), 50 μ m. (F) High-power view of spinous layer in normal epidermis with intercellular spines visible, overlying granular layer with purple keratohyalin granules and basket weave stratum corneum. (G) High-power view of affected skin shows perinuclear vacuolization (black arrows), lack of keratohyalin granules, and retained nuclei (white arrows) in the stratum corneum. (H) High-power view of revertant skin shows normal spinous layer with intercellular spines, granular layer with purple keratohyalin granules in keratinocytes, and basket-weave stratum corneum. Scale bars in (F) to (H), 25 μ m.

confined to the interval from 21.7 Mb (near the centromere) to 34.5 Mb.

These observations suggest that IWC is genetically homogeneous and localize the disease locus to a 99.9% confidence interval, calculated from the position of the most distal recombinant and the number of independent recombinants, to the 34.5 to 37.7 Mb interval on 17q. This interval is notable for a gene cluster encoding 28 type-1 keratins and 24 keratin-associated proteins (5).

Assuming that affected offspring of unaffected parents harbor *de novo* mutations in the IWC gene, we conducted Illumina sequencing of overlapping polymerase chain reaction (PCR) amplicons spanning the entire critical interval in a parent-offspring trio. At mean 95× per base coverage, ~95% of all the bases in the 99.9% confidence interval were read at least 10 times in each subject, which enabled high-quality genotype calls. The affected subject had a single *de novo* mutation (table S1), which was confirmed by Sanger sequencing (Fig. 3A). The mutation abolishes the canonical splice acceptor site of intron 6 of *keratin 10* (*KRT10*) (TAG to TGG mutation). Moreover, the mutation was absent in revertant spots (fig. S3). Sequencing of *KRT10* transcripts from mutant keratinocyte cDNA revealed a wild-type and a mutant isoform showing splicing at an AG site at bases 7 to 8 in the normal exon 7, leading to an eight-base deletion (Fig. 3, B and C). This results in a frameshift at normal codon 458, leading to 119 aberrant amino acids followed by termination at codon 577. The frameshift peptide has an extremely skewed amino acid composition, with 67 arginine residues (Fig. 3D).

Sequencing of *KRT10* from genomic DNA and disease keratinocyte cDNA in the six other IWC kindreds identified *de novo* mutations in all four simplex kindreds and transmitted mutations

in the two multiplex kindreds (table S2; Fig. 3, C and D; and fig. S4). It is noteworthy that all mutations resulted in cDNAs encoding frameshifts that enter the same alternative C-terminal reading frame (Fig. 3, C and D, and fig. S5). Mutations included two additional intron 6 splice acceptor mutations, an intron 6 splice donor site mutation that results in skipping of exon 6, two frameshift mutations in exon 7, and an exon 6 mutation that creates a premature splice donor site. All of these mutations are absent among control chromosomes, and each is lost in revertant spots (fig. S3). On the basis of these findings, we conclude that mutations in *KRT10* cause IWC.

K10 is highly expressed in the suprabasal layers of the epidermis and forms heterodimers with keratin 1 (6, 7), which then assemble to form 10-nm intermediate filaments (8). In diseased skin, keratin 10 levels are reduced (fig. S6, A and B), and electron microscopy reveals a marked reduction in the total number of cytokeratin filaments and poor investment of desmosomes with filaments (fig. S7). In addition, however, K10 is also mislocalized in diseased skin, with prominent nuclear localization in discrete foci that prove to be nucleoli when costained with fibrillarin (Fig. 4, A to F); this nuclear localization is not seen in normal or revertant skin. Keratin 1 shows similar mislocalization in diseased skin (fig. S6, C to E). Similarly, although wild-type and C-terminal-truncated K10 localize to the cytoplasmic filament network when expressed in PLC cells, K10 harboring disease-causing frameshifts localizes exclusively to the nucleolus (Fig. 4, G to L).

In summary, we demonstrate that IWC is associated with dominant mutations in *keratin 10*, all of which produce an arginine-rich C-terminal peptide that causes mislocalization of the protein to the nucleolus. This mislocalization provides a

mechanism for disruption of the keratin filament network, which in turn contributes to loss of barrier function. The observed abnormalities in differentiation are unlikely to be due simply to loss of the keratin network, and we speculate that the mutant K10 may disrupt cellular physiology in additional ways, perhaps through effects on ribosomal biogenesis or cell cycle regulation, a process to which K10 has been linked (9). We hypothesize that the nucleolar localization of mutant K10 may be due to RNA binding, owing to the extremely arginine-rich frameshift peptide and the high concentration of ribosomal RNA in the ribosome assembly factory. Most RNA-binding proteins have arginine-rich motifs that interact with the phosphate backbone of RNA, and the specific arginine-rich sequences capable of binding to RNA are diverse (10, 11). Similarly, arginine-rich motifs also contribute to nuclear localization (12). Although we have not seen localization of the frameshift peptide to other RNA- or DNA-containing structures, we cannot exclude this possibility.

IWC is remarkable for its high frequency of spontaneous reversion, with more than a thousand revertants in many subjects. The mechanism—mitotic recombination—represents the complement of a mechanism for producing somatic homozygosity for tumor suppressor mutations (13–15). The revertants in IWC are clonal, detectable in the first year of life, and widely distributed in both sun-exposed and unexposed skin. These recombination events simultaneously create homozygous mutant cells, but we see no phenotypic evidence of these, which suggests that they are lethal to cells or contribute little to the epidermal surface.

Somatic reversion has previously been reported for several other disorders (16). These include recessive diseases such as Bloom syndrome and Fanconi anemia and X-linked Wiscott-Aldrich syndrome in which 10 to 20% of patients show some revertant blood cells (17–19). These revertant blood cell clones arise by various mechanisms, including intragenic recombination, gene conversion, second-site complementation, and direct reversion. In Bloom syndrome, these revertant clones contributed to refined mapping of the disease locus, analogous to the mapping approach used herein (20).

Similarly, mutations underlying a substantial number of other severe dominant skin diseases have been identified, including keratitis-ichthyosis-deafness syndrome, progressive symmetric erythrorokeratoderma, ichthyosis bullosa of Siemens, and dominant dystrophic epidermolysis bullosa; to our knowledge, only a single revertant clone, produced by second-site complementation, has been reported for these diseases (21). Similarly, a fraction of patients with recessive skin disorders have been reported to have revertant patches of skin comprising one to six reported patches in a total of seven patients. In cases where the mechanism was established, all arose by second-site mutation or gene conversion (22–27). Moreover, no revertant clones have been reported, or seen in our clinics, in patients with dominant negative or

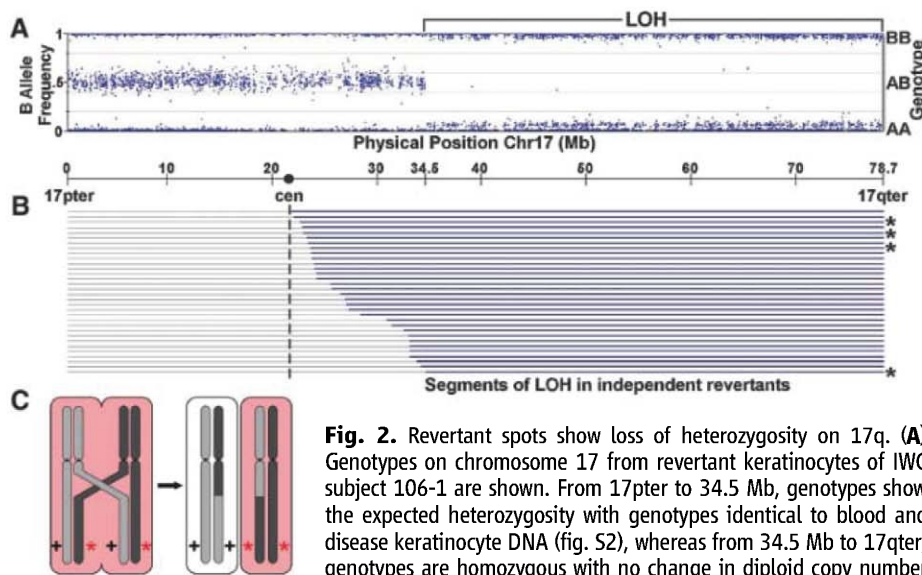


Fig. 2. Revertant spots show loss of heterozygosity on 17q. (A) Genotypes on chromosome 17 from revertant keratinocytes of IWC subject 106-1 are shown. From 17pter to 34.5 Mb, genotypes show the expected heterozygosity with genotypes identical to blood and disease keratinocyte DNA (fig. S2), whereas from 34.5 Mb to 17qter, genotypes are homozygous with no change in diploid copy number (fig. S2). (B) Results of genotyping keratinocytes of 32 revertant spots

from seven unrelated IWC subjects (106-1 revertants denoted with an asterisk). Gray lines, genomic segments with heterozygous genotypes matching blood DNA; blue lines, segments showing copy-neutral LOH. These results are consistent with the hypothesis that IWCs are caused by a dominant allele distal to 34.5 Mb that is lost by mitotic recombination, as depicted in (C).

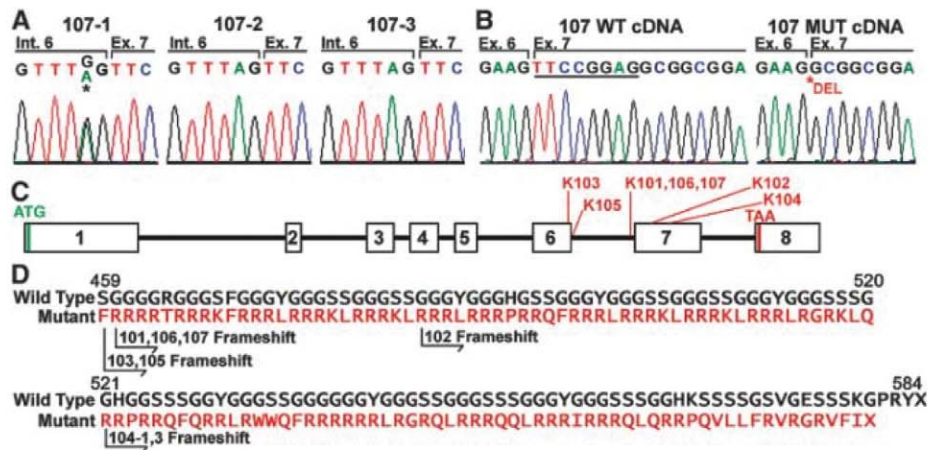


Fig. 3. Mutations in *KRT10* (keratin 10) are associated with IWC. **(A)** Sanger sequencing confirms de novo mutation in *KRT10* from Illumina sequencing that is absent in the parents (107-2 and 107-3) but present in the affected offspring (107-1) that abolishes the splice acceptor site of intron 6. **(B)** Abnormal splicing of *KRT10*. cDNA from diseased keratinocytes of 107-1 shows two splice forms, one wild-type (WT) and one using an AG splice acceptor that deletes eight bases from WT cDNA (underlined). **(C)** The genomic structure of *KRT10* is shown. The locations of mutations found in IWC kindreds are indicated. **(D)** IWC frameshifts all produce an arginine-rich C-terminal peptide. The normal sequence of the C-terminal 226 amino acids of keratin 10 is shown; below, in red, the sequence of the frameshift peptides found in IWC are shown, with the position of the frameshift in each kindred indicated.

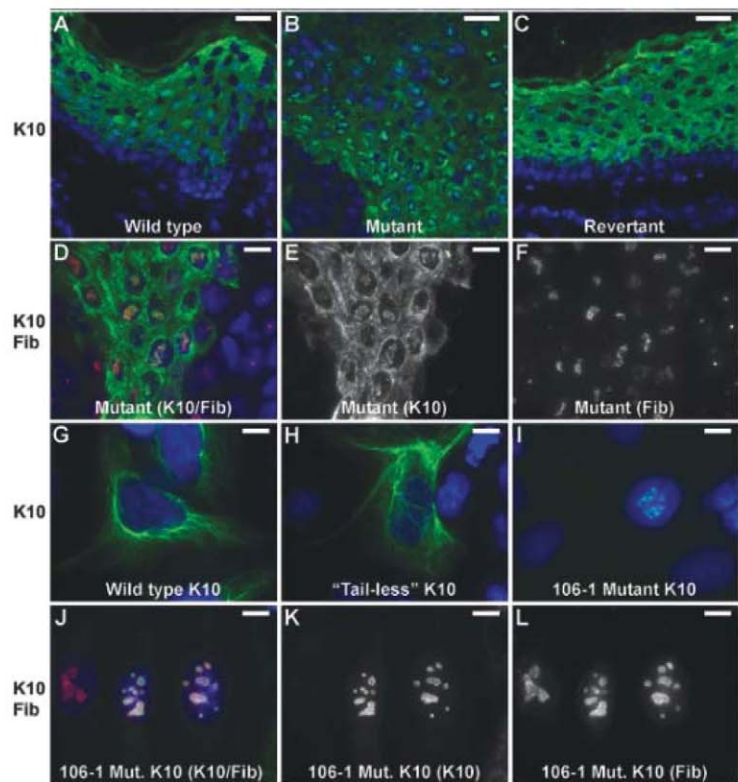


Fig. 4. Mutant keratin 10 is redirected to nucleoli in vivo and in vitro. **(A to C)** Images of normal, mutant, and revertant skin stained with 4',6'-diamidino-2-phenylindole (DAPI) and antibodies to keratin 10 reveal discrete foci of nuclear keratin 10 in mutant skin which are absent in normal and revertant skin. Scale bars, 50 μ m. **(D to F)** Costaining with the nucleolar marker fibrillarin shows that keratin 10 is in the nucleolus. Scale bars, 10 μ m. **(G to I)** Constructs bearing wild-type keratin 10 (**G**), keratin 10 truncated at the beginning of the tail domain (amino acid 459) (**H**), and keratin 10 with the kindred 106 frameshift mutation beginning at codon 460 (**I**) were expressed in human hepatoma PLC cells and stained with DAPI and monoclonal antibody to keratin 10. Wild-type and "tailless" K10 integrate into the cytoplasmic filament network, whereas the IWC mutant K10 localized to nucleoli as shown by costaining with fibrillarin (**J** to **L**). Scale bars (**J**) to (**L**), 10 μ m.

recessive mutations in keratin 10 that cause a distinct disease, epidermolytic ichthyosis (also known as epidermolytic hyperkeratosis) (fig. S8) (28). These exceptions underscore the infrequency of spontaneous reversion and the generally low frequency of mitotic recombination as a mechanism of reversion. In particular, the absence of reversion of other dominant missense mutations in *KRT10* implicates the IWC frameshift mutations in the appearance of revertant clones.

The high frequency of somatic reversion in patients with IWC suggests that revertant stem cell clones are under strong positive selection and/or that the rate of production of revertant clones is markedly elevated. The persistence of revertant clones indicates that the reversion event must occur in epidermal stem cells. Epidermal stem cell units have been estimated to populate a fixed area of approximately 0.25 to 0.5 mm² in human skin (29, 30). The revertant clones we observe in adults with IWC increase in size with time and reach up to 4 cm, consistent with positive selection. Nonetheless, rare revertants in other skin diseases have achieved very large size (22–27), from which it may be argued that positive selection is not likely the sole rate-limiting step in production of detectable revertants; however, it suggests an increased rate of mitotic recombination in IWC. Similarly, the fact that none of the previously described revertants in other skin diseases, but all of the IWC revertants, have occurred via mitotic recombination lends support to an effect on the rate of mitotic recombination.

Both mechanisms would be most readily explained by effects of the mutant peptide in epidermal stem cells: Toxic effects could give revertants a survival or replicative advantage; effects on DNA replication, repair, or cell cycle could also promote mitotic recombination. Although K10 is classically regarded as an early differentiation marker, there is evidence that a small proportion of basal cells, which contain rare epidermal stem cells, express *KRT10*. Moreover, purified putative stem cells of the interfollicular epidermis and follicular bulge show substantial *KRT10* expression in proliferating cells, which supports this possibility (30–32).

Genetic therapies for dominant diseases have focused on correction of mutations (33) or inhibition of mutant protein synthesis by antisense or interfering RNAs (34). Our results raise the possibility that induction and/or selection of mitotic recombination could be exploited for therapeutic benefit to accomplish cellular reversion of other disease-causing mutations. In this scenario, however, the potential adverse effects of producing homozygosity at undesired loci across the genome would have to be carefully considered in conjunction with the potential benefit of reversion.

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Greater Neural Pattern Similarity Across Repetitions Is Associated with Better Memory

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Repeated study improves memory, but the underlying neural mechanisms of this improvement are not well understood. Using functional magnetic resonance imaging and representational similarity analysis of brain activity, we found that, compared with forgotten items, subsequently remembered faces and words showed greater similarity in neural activation across multiple study in many brain regions, including (but not limited to) the regions whose mean activities were correlated with subsequent memory. This result addresses a longstanding debate in the study of memory by showing that successful episodic memory encoding occurs when the same neural representations are more precisely reactivated across study episodes, rather than when patterns of activation are more variable across time.

Repeated study of the same materials can significantly strengthen memory representations and make them more resistant to forgetting (*1*), but not all repetitions are equal. One fundamental issue is how multiple study episodes add up to improve later memory. A widely accepted theory, often referred to as the encoding variability hypothesis (*2–5*), proposes that each study episode is encoded differently as a result of contextual drift with time, and that greater encoding variability leads to better mem-

ory. Alternatively, it has been claimed that each subsequent study episode serves as a retrieval cue to reactivate and strengthen the memory representation of the information stored during earlier study episodes (*6*). Evidence for this reactivation view comes from the finding in an AB-AC paradigm in which the presence of A during AC study reinstated AB and therefore also improved memory for B (*7*), an effect related to activity in the posterior medial temporal lobe (*8*). However, previous work has not yet established a link between the nature of neural representations during encoding and later memory.

In this study, we used functional magnetic resonance imaging (fMRI) and representational similarity analysis (*9*) to examine how the similarity in patterns of neural activity across multiple study presentations is related to subsequent memory. The encoding variability hypothesis predicts that better subsequent memory should be associated with greater dissimilarity between

activity patterns across study presentations. To the contrary, we found, across three studies, that better subsequent recognition and recall is associated with greater similarity between neural activity patterns across repetitions, consistent with the hypothesis that practice improves memory by retrieving and strengthening a consistent representation.

In the first experiment, 24 subjects were scanned while memorizing 120 novel faces (Fig. 1A) (*10*). Each face was presented four times, with an interrepetition interval (ITI) ranging from 1 (i.e., consecutive) to 20 faces. One hour after the scan, subjects were given a recognition memory test, during which a total of 240 faces (half learned, half new) were randomly mixed together. For each stimulus, the subjects had to decide whether or not it had been presented before by responding on a 6-point confidence scale, from 1 (definitely new) to 6 (definitely old). Out of the 120 old faces, subjects on average recognized with high confidence (e.g., a 5 or 6 rating) 51.7 ± 18.6 items and forgot (a 1 or 2 rating) 37.3 ± 16.9 items (table S1). Using a subsequent memory paradigm (*11, 12*), we compared encoding-related brain activity for subsequently recognized faces with that for subsequently forgotten faces across four repetitions. Consistent with previous literature (*13–15*), this comparison identified stronger activation for subsequently remembered faces than for subsequently forgotten faces in the left fusiform gyrus (Montreal Neurological Institute, MNI: $-46, -60, -8, Z = 3.88$) and right fusiform gyrus (MNI, $44, -60, -10, Z = 3.58$), extending into the inferior temporal gyrus and lateral occipital cortex (fig. S1).

We then tested the core hypothesis that pattern similarity in this region is associated with subsequent memory. To do this, we reestimated the model with unsmoothed data.

We then extracted the signal for each individual voxel within anatomically defined re-

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gions of interest (ROIs) and used representational similarity analysis (9) to examine the degree of similarity in the fMRI activation patterns between repetitions (averaged over stimuli), using the Pearson correlation coefficient as the similarity metric. Because pattern similarity can be affected by the number of trials in each condition (fig. S2), our analyses were based on a model that matched the number of trials included in the regressors for remembered and forgotten items, as well as their repetition lags.

We focused our analyses on 20 independent anatomically defined regions in the dorsal and ventral visual stream, frontoparietal cortex, and middle and medial temporal cortex (table S3), all of which have been previously shown to be important for visual object perception and memory encoding. Nine of these regions showed significantly higher degrees of pattern similarity for subsequently remembered faces than subsequently forgotten faces ($P < 0.05$, two regions remained significant by Bonferroni correction), whereas no regions showed the opposite effect (Figs. 2 and 3, fig. S3, and table S4). Only four regions—the left inferior frontal gyrus, right inferior frontal gyrus, right fusiform gyrus, and right parahippocampal gyrus (LIFG, RIFG, RFUS, and RPHG, respectively)—showed stronger overall activation for subsequently remembered faces than subsequently forgotten faces (table S4). To ensure that the significantly higher pattern similarity was not caused by differences in mean activity, we reran this analysis in the bilateral ventral visual stream—the lateral occipital lobe, fusiform gyrus, and inferior temporal gyrus (LOC, FUS, and ITG, respectively)—after removing voxels showing significant subsequent memory effects in mean activity under a liberal threshold ($P < 0.05$, uncorrected). Even after removing these mean-responsive voxels, there was a significantly higher degree of pattern similarity for remembered versus forgotten faces ($F_{1,23} = 6.16$, $P = 0.02$) (Fig. 3 and table S4).

The results from our first experiment suggest that the degree of pattern similarity between successive study episodes is associated with subsequent memory performance in a recognition test. Because free recall is more sensitive to contextual associations than recognition is, the encoding variability hypothesis thus predicts that subsequently recalled items might be associated with more divergent contexts than items that are not subsequently recalled (16–18). To provide a further and more direct test of the encoding variability hypothesis, we conducted a second experiment to examine whether greater pattern similarity is also associated with better free-recall performance. Subjects ($n = 22$) were asked to perform a semantic (concrete versus abstract) judgment task on familiar words during the scan (10). Each word was repeated three times, with a repetition lag ranging from 1 to 18 trials. After the study session, participants were asked to return 6 hours later to perform two memory tests. In the first test, subjects were asked to recall the words they had

studied in the scanner. They were then asked to perform a recognition test similar to that described in Experiment 1. Out of the 180 items, 44.7 ± 21.7 items were categorized as Recalled (items correctly recalled on the free-recall test), 81.2 ± 25.5 items as Recognized [items recognized with high confidence (score of 5 or 6) but not recalled], and 54.1 ± 27.4 items as Forgotten (items that were neither recalled nor recognized) (table S1).

The differences in behavioral performance and mean activation during the semantic task among recalled, recognized, and forgotten items are depicted in table S2 and fig. S4. From the

findings from Experiment 1, we constructed a new model using equal numbers of trials from the recalled, recognized, and forgotten conditions and calculated the pattern similarity in the same 20 regions. We found that out of the 20 regions, 15 regions showed a higher level of pattern similarity across repetitions (again averaging over items) for subsequently recalled items than for subsequently recognized or forgotten items (one region remained significant by Bonferroni correction); no region showed the opposite effect (fig. S5 and table S5). Only four regions showed stronger mean activation for subsequent recalled

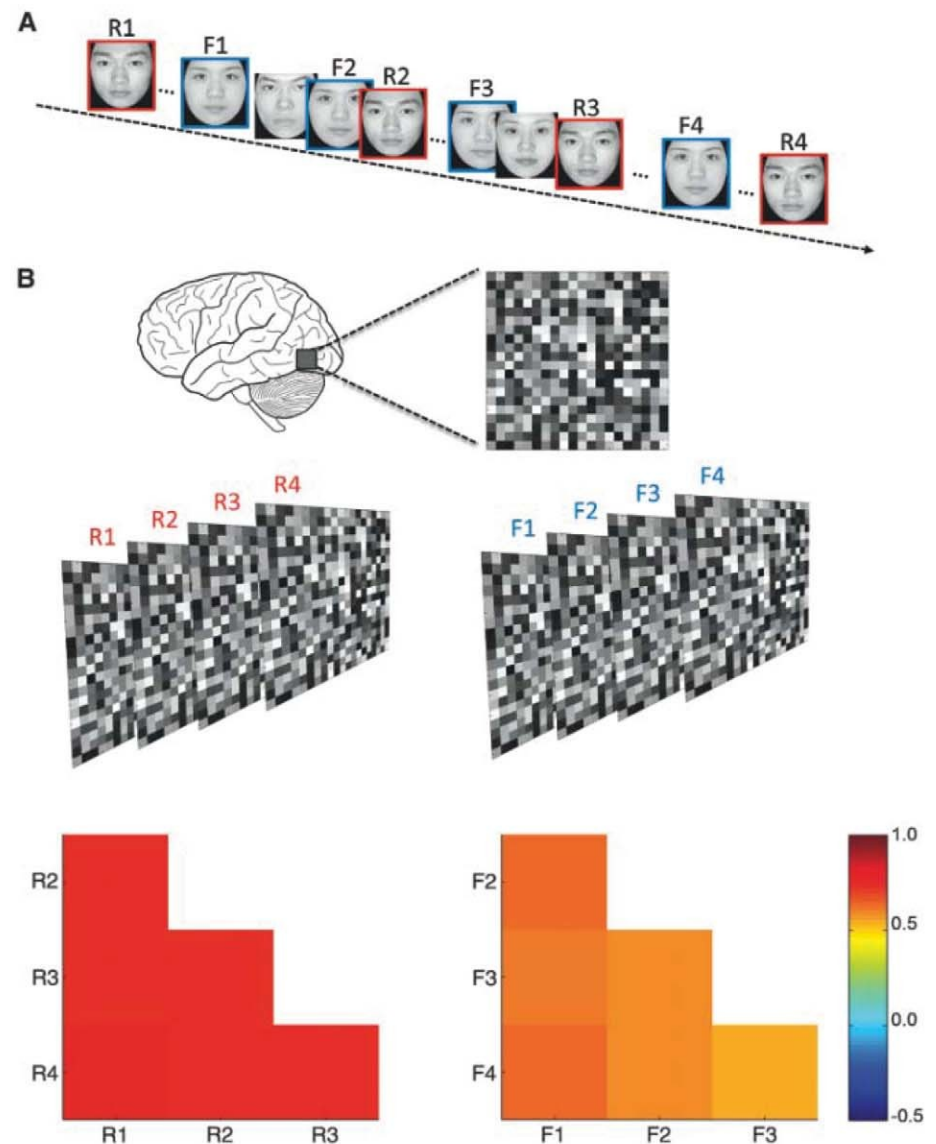


Fig. 1. (A) Experimental design of Experiment 1 and (B) schema of the cross-repetition pattern analysis. A total of 120 novel faces were studied over three scanning runs. (A) Each face was repeated four times. They were categorized post hoc, as remembered faces and forgotten faces, according to performance on the recognition memory test administered after a 1-hour delay. Each presentation of the remembered faces (R1 to R4) and forgotten faces (F1 to F4) was separately modeled. (B) Pattern analysis was based on independent structural ROIs (top) (10). Activation pattern in a given ROI was extracted for each presentation (middle) and then subjected to Pearson correlation analysis. The encoding variability hypothesis predicts that the degree of pattern similarity for subsequently remembered faces is lower than that for subsequently forgotten faces (bottom).

words than for subsequently forgotten words (table S5). Again, after removing voxels showing subsequent memory effects for mean activation ($P < 0.05$, uncorrected), the remaining voxels in the left dorsal visual stream [dorsal lateral occipital lobe (dLOC) and inferior parietal lobe

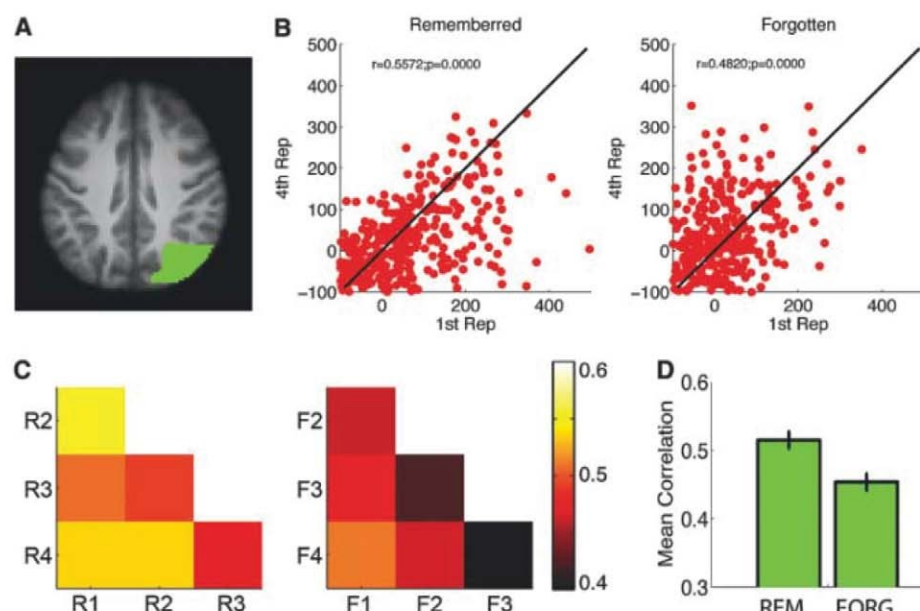


Fig. 2. Neural pattern similarity in a sample region. **(A)** The location of the right dorsal lateral occipital cortex (RdLOC), which was anatomically defined according to the Harvard-Oxford probabilistic map, and overlaid onto the group-averaged anatomical map. **(B)** Neural pattern similarity from a single subject's single-run data. Pattern similarity was calculated by computing the correlation between the parametric estimates (beta) for each voxel within the ROI across the two repetitions. The line reflects unit slope. **(C)** Neural pattern similarity averaged across all subjects ($n = 24$), separately for each pair of a repetition combination. **(D)** The mean neural pattern similarity as a function of subsequent memory. A repeated-measures analysis of variance (ANOVA) was used to examine the differences between conditions. Error bars represent within-subject error. REM, remembered; FORG, forgotten.

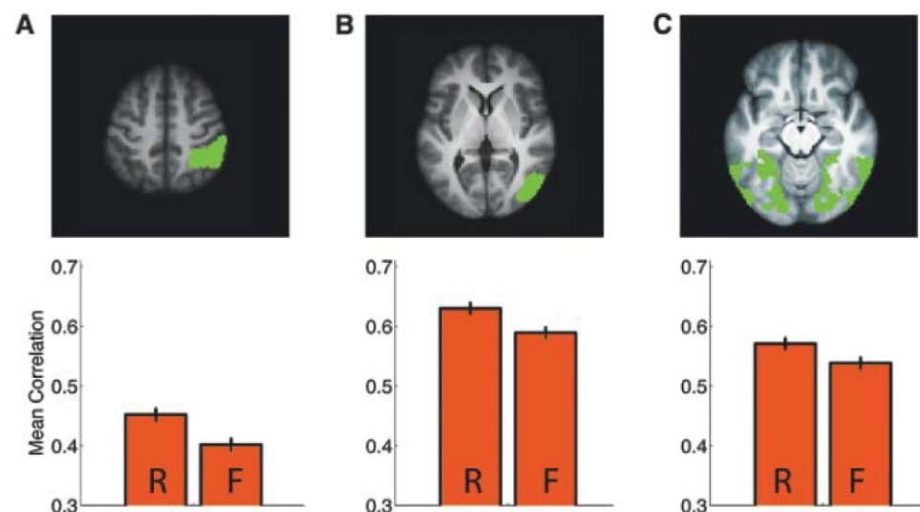


Fig. 3. Neural pattern similarity is associated with face memory. Greater pattern similarity for subsequently remembered faces than for subsequently forgotten faces was found in **(A)** the right inferior parietal lobule (RIPL), **(B)** the right ventral lateral occipital cortex (RvLOC), which were anatomically defined, and **(C)** the bilateral ventral visual cortex, which includes the bilateral fusiform gyrus, bilateral inferior temporal gyrus and bilateral ventral lateral occipital cortex, but excludes voxels showing significant subsequent memory effects in activation levels defined by a liberal threshold ($P < 0.05$, uncorrected). All ROIs were overlaid onto the group-averaged anatomical map. The bar graphs show the group-averaged ($n = 24$) mean correlation of all pairs of repetitions as a function of subsequent memory. Error bars represent within-subject error. R, remembered; F, forgotten; See table S4 and fig. S3 for detailed statistics and results for other regions.

(IPL)] also showed greater pattern similarity across repetitions for subsequently recalled than subsequently recognized or forgotten words ($F_{1,21} = 4.64$, $P = 0.015$) (fig. S5 and table S5).

In both Experiments 1 and 2, the use of rapid event-related designs did not allow for reliable estimates of item-specific blood oxygen level-dependent (BOLD) activation patterns, and pattern similarity was calculated from the aggregated BOLD activation pattern across stimuli in each condition. There are thus two possible explanations that are consistent with these results. First, it is possible that the results reflect overlap in item-level encoding processes. Alternatively, it is possible that they reflect process-level overlap, such that better memory occurs when the same general processes (e.g., perceptual, attentional, or semantic processes) are engaged across repetitions. In order to more directly test the hypothesis of item-specific pattern overlap, we performed a third experiment using a slow event-related fMRI design (12 s for each trial), which enabled us to extract BOLD signal patterns associated with each single trial. In this experiment, 22 young adults were asked to perform a semantic (living versus non-living) judgment task on 60 familiar words during the scan (10). To prevent further encoding of each item during the repetition lag, subjects performed a highly engaging self-paced visual orientation judgment task for 8 s after each semantic judgment task (lasting for 3 s), and the next trial started after a 1-s delay (fig. S6). Each item was repeated three times, with a repetition lag ranging from four to nine trials. Thirty minutes after the study session, participants were asked to freely recall the words they had studied in the scanner. Out of the 60 items, subjects, on average, recalled 13.5 ± 4.7 items (table S1).

Subjects' response time on the semantic judgment task decreased across repetitions ($F_{2,42} = 42.96$, $P < 0.001$); accuracy was high (mean = $97.5\% \pm 2\%$) and did not change across repetitions ($F_{2,42} = 1.68$, $P = 0.19$). Accuracy ($F_{1,21} = 1.79$, $P = 0.19$) and response times ($F_{1,21} = 0.15$, $P = 0.70$) did not differ between subsequently recalled items and forgotten items (table S2). There were no significant interactions between repetition and subsequent memory for either accuracy ($F_{2,42} = 0.14$, $P = 0.087$) or response time ($F_{2,42} = 1.69$, $P = 0.20$). Functional imaging data revealed that, similarly to Experiment 2, there were significantly stronger activations for subsequently recalled items than for subsequently forgotten items in the left middle and inferior frontal gyrus (LMFG/LIFG) (MNI: $-50, 14, 34$; $Z = 4.17$), and the left dorsal lateral occipital lobe (LdLOC) and adjacent inferior parietal lobule (LIPL) (MNI: $-40, -66, 46$; $Z = 3.48$) (fig. S7).

We then examined whether the degree of pattern similarity in the 20 anatomically defined regions was associated with subsequent memory performance. We constructed a beta-series model (19) with one regressor for each trial and estimated the model using ridge regression (20). Consistent with the first two experiments, 7 of the

20 regions showed a significantly higher level of pattern similarity across repetitions for subsequently recalled items than for subsequently forgotten items ($P < 0.05$; one region remained significant by Bonferroni correction), whereas no region showed the opposite effect (Fig. 4, fig. S8, and table S6). Taking the LdLOC as an example, we found that the level of pattern similarity across repetitions showed a significant subsequent memory effect ($F_{1,21} = 18.69$, $P = 0.0003$) (Fig. 4D). Again, after removing voxels showing subsequent memory effects in terms of activation levels ($P < 0.05$, uncorrected), the remaining voxels in the left dorsal visual stream (dLOC and IPL) also showed stronger pattern similarity across repetitions for subsequently recalled than subsequently recognized or forgotten words ($F_{1,21} = 7.97$, $P = 0.01$) (Fig. 4F and table S6).

Finally, if the degree of pattern similarity truly reflects item-specific reinstatement of activation patterns, we should expect a higher level of pattern similarity within items (i.e., cross-repetitions) than between items. To test this prediction, we calculated the averaged across-item pattern similarity of all possible pairings (except the within-item, cross-repetition pairings), separately for recalled items and forgotten items. The results showed that within-item correlation was higher than that for cross-item correlation, especially for recalled items (fig. S8 and table S7). Again taking the LdLOC as an example, we found that the degree of pattern similarity across repetitions in this region for recalled items was significantly higher than cross-item pattern similarity [$t(21) = 3.13$, $P = 0.005$], whereas the difference was not significant for forgotten items ($P = 0.41$) (Fig. 4D), which suggests that repeatedly studying the same material is not sufficient to introduce activation reinstatement at the item-specific level, and failure of pattern reinstatement is associated with forgetting.

We took a number of measures to ensure that our results were not due to the effects of rep-

etition lag or repetition priming. In addition to matching the number of trials, we also carefully matched the repetition lags between remembered and forgotten items (Experiment 1: remembered versus forgotten: 6.11 versus 6.05; $t = 1.02$, $P = 0.31$; Experiment 2: recalled versus recognized versus forgotten: 6.03 versus 6.02 versus 5.63, $F_{2,42} = 1.41$, $P = 0.26$). There was also no difference in the repetition lag between recalled and forgotten trials in Experiment 3 (6.4 versus 6.3, $t = 1.22$, $P = 0.23$). In addition, we did not find a significant interaction between repetition priming and subsequent memory in most of the brain regions in any of the experiments (tables S4 to S6). Third, in Experiment 3, the reaction times and accuracy in the semantic judgment task and the orientation judgment task that followed subsequently remembered and forgotten items were not different (table S2). Finally, for Experiments 1 and 2, the degree of pattern similarity is not an artifact of design matrix orthogonality (figs. S9 and S10).

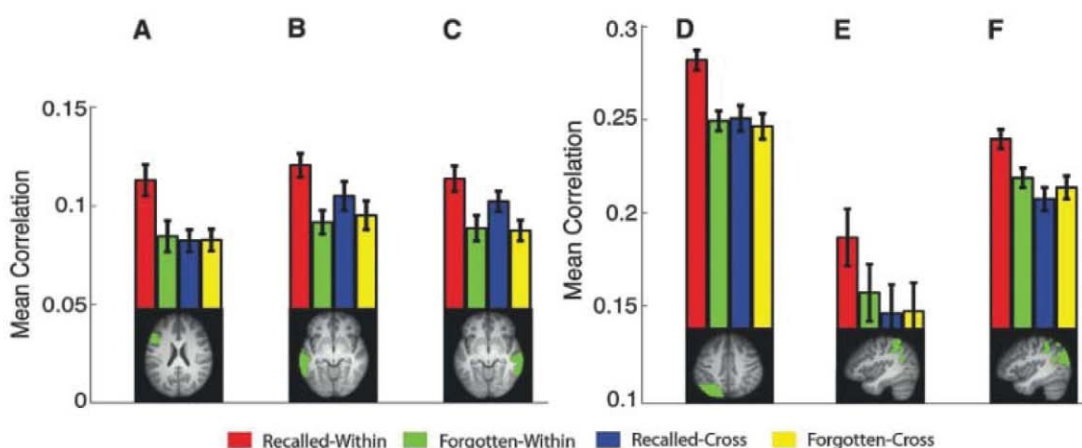
Taken together, our results suggest that episodic memory encoding is enhanced by reactivating the initial neural representation in each subsequent study episode. Using different study materials and different memory tests, our data suggest that pattern reinstatement can account for subsequent memory effects for both verbal and nonverbal materials and in both recall and recognition tests. Although the within- versus across-items analysis in Experiment 3 demonstrates a significant effect of pattern overlap at the level of individual items, the results of Experiments 1 and 2 are also consistent with an effect of process-level overlap; we propose that both of these are likely to be important in determining the effectiveness of repeated study. We suggest that repeated study episodes lead to more effective encoding when the same neural representation is reinstated, which is incompatible with the encoding variability hypothesis. Consistent with our results, it has recently been shown that more re-

producible neural patterns are associated with more conscious cognitive processing (21), which suggests that consistency of pattern engagement may be a more general marker of effective cognitive processing (perhaps because of its effects on memory encoding).

Previous studies have shown that memory retrieval is associated with reactivation of some of the same sensory regions that were activated during perception of those items (22–26). This category-specific or sequence-specific activation reinstatement precedes memory (27) and is associated with performance in free recall (28). The present study extends these findings and shows that during subsequent learning where no explicit retrieval was required, item-specific pattern reinstatement occurs, resulting in a stronger episodic encoding event that supports subsequent memory. Our results are also consistent with evidence showing that memory consolidation, whether during sleep or awake periods following learning, involves replay of neural activation patterns during learning (29, 30). Given the important role of memory retrieval on memory retention (31), these results suggest that reactivation of the same neural pattern during initial learning, whether during repeated practice, memory consolidation, and/or memory retrieval, can enhance memory.

Although most previous studies on the subsequent memory effect focused on one-shot learning, real learning in daily life often involves repeated practice. Our study suggests that, for repeated study events, pattern reinstatement is as sensitive as, if not more sensitive than, overall activation (11, 12, 32) as a predictor of subsequent memory. Our approach can readily be used to examine the neural mechanisms underlying other manipulations that affect memory encoding during repeated practice, such as the spacing effect and the variance effect (7), which would help to clarify the effects of encoding variability. However, fMRI data are a relatively coarse aggregate measure of the responses of large popula-

Fig. 4. Neural pattern similarity is associated with free recall of words. Greater pattern similarity for subsequently recalled words than for forgotten words was found in (A) the LIFG, (B) the left middle temporal gyrus (LMTG), (C) the right middle temporal gyrus (RMTG), (D) the LdLOC, (E) LIPL, and (F) the left dorsal visual stream that includes the anatomical region of LIPL and LdLOC, but excludes voxels showing significant subsequent memory effects in activation levels when we assume a liberal threshold ($P < 0.05$, uncorrected). All ROIs were overlaid onto the group-averaged anatomic map. The bar graphs show the group-averaged ($n = 22$) mean correlation as a function of subsequent memory. The within-item correlation was calculated for each individual item (averaged across all pairs of repetitions) and then averaged separately for



recalled and forgotten items. The cross-items correlation was calculated between items within each memory status. Error bars represent within-subject error. See tables S6 and S7 and fig. S8 for detailed statistics and results for other regions.

tions of neurons and, thus, may not necessarily capture all of the aspects of encoding variability that might be at play. Future studies need to further examine this issue by applying similar approaches using complementary neuroimaging techniques, such as electroencephalography and single-unit recording.

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Supporting Online Material

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The CRAC Channel Activator STIM1 Binds and Inhibits L-Type Voltage-Gated Calcium Channels

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Voltage- and store-operated calcium (Ca^{2+}) channels are the major routes of Ca^{2+} entry in mammalian cells, but little is known about how cells coordinate the activity of these channels to generate coherent calcium signals. We found that STIM1 (stromal interaction molecule 1), the main activator of store-operated Ca^{2+} channels, directly suppresses depolarization-induced opening of the voltage-gated Ca^{2+} channel $\text{Ca}_v1.2$. STIM1 binds to the C terminus of $\text{Ca}_v1.2$ through its Ca^{2+} release-activated Ca^{2+} activation domain, acutely inhibits gating, and causes long-term internalization of the channel from the membrane. This establishes a previously unknown function for STIM1 and provides a molecular mechanism to explain the reciprocal regulation of these two channels in cells.

Excitable and nonexcitable cells are distinguished by their ability to increase their concentration of intracellular calcium ($[\text{Ca}^{2+}]_i$) in response to membrane depolarization (1). Excitable cells such as neurons and myocytes have voltage-gated Ca^{2+} channels (VGCCs) that are activated by depolarization and are essential for synaptic vesicle release, contraction, and electrical excitability (2–4). In contrast, nonexcitable cells such as lymphocytes and mast cells lack voltage-gated Ca^{2+} influx but have Ca^{2+} release-activated Ca^{2+} (CRAC) channels (5–7). These are activated by receptors that deplete the internal Ca^{2+} stores and are important for regulating gene expression

and controlling cell proliferation and differentiation (5). Excitable cells express store-operated Ca^{2+} channel proteins, but these contribute little to Ca^{2+} influx (8, 9), whereas nonexcitable cells express VGCC proteins but lack voltage-gated Ca^{2+} currents (10, 11). The underlying mechanisms that account for the reciprocal regulation of these Ca^{2+} influx pathways are not understood.

VGCCs are composed of an $\alpha 1$ subunit that contains the pore and voltage sensor of the channel, and β and $\alpha 2\delta$ subunits that modulate trafficking and gating (12, 13). The $\alpha 1$ subunit has four repeats of six transmembrane domains and cytoplasmic N and C termini (14). L-type Ca^{2+} channels (LTCs) have a large single-channel conductance, are sensitive to dihydropyridine blockers, and are encoded by the Ca_v1 family of $\alpha 1$ subunits (15). $\text{Ca}_v1.2$ channels are the most abundant LTCs in the heart and brain and are essential for cardiac contraction and for neuronal function (16).

CRAC channels are composed of STIM (17–19) and Orai proteins (20–22). STIM1 is a single-pass endoplasmic reticulum protein with an intraluminal EF hand and cytoplasmic coiled-coil and lysine-rich domains (17–19). Upon depletion of the Ca^{2+} stores, STIM1 forms oligomers that translocate to endoplasmic reticulum–plasma membrane junctions and bind to Orai channels at the plasma membrane (17, 23). STIM1 binds to Orai via a cytoplasmic region called the CRAC activation domain (CAD) that is both necessary and sufficient for channel opening (24, 25).

Rat cortical neurons express LTCs that generate a $[\text{Ca}^{2+}]_i$ rise after membrane depolarization (Fig. 1A). These cells show little store-operated Ca^{2+} influx, as treatment of the cells with 1 μM thapsigargin (TG) to deplete the internal stores does not cause a $[\text{Ca}^{2+}]_i$ rise (Fig. 1A). To test whether depletion of stores affects voltage-gated Ca^{2+} channels, we treated cells with 1 μM TG and then stimulated the cells with a depolarizing pulse of KCl (Fig. 1, B and C). Treatment with TG led to a 21% decrease in the initial slope of the $[\text{Ca}^{2+}]_i$ rise, which suggests that depletion of the internal stores inhibited the conductance by VGCCs. Because the slope of the $[\text{Ca}^{2+}]_i$ rise reflects sources of Ca^{2+} other than plasma membrane Ca^{2+} channels, we used whole-cell patch clamping to measure LTC activity directly. We transfected human embryonic kidney (HEK) 293 cells with $\text{Ca}_v1.2$, $\alpha 2\delta$, and $\beta 1b$ subunits and used whole-cell patch clamping to measure the $\text{Ca}_v1.2$ currents. Treatment of these cells with TG led to a 15% decrease in the amplitude of the $\text{Ca}_v1.2$ currents over a period of 200 s, consistent with the idea that depletion of stores inhibits $\text{Ca}_v1.2$ channels (Fig. 1D). Depletion of the stores did not alter the current-voltage (I - V) relationship or the inactivation of the channels. To test whether inhibition

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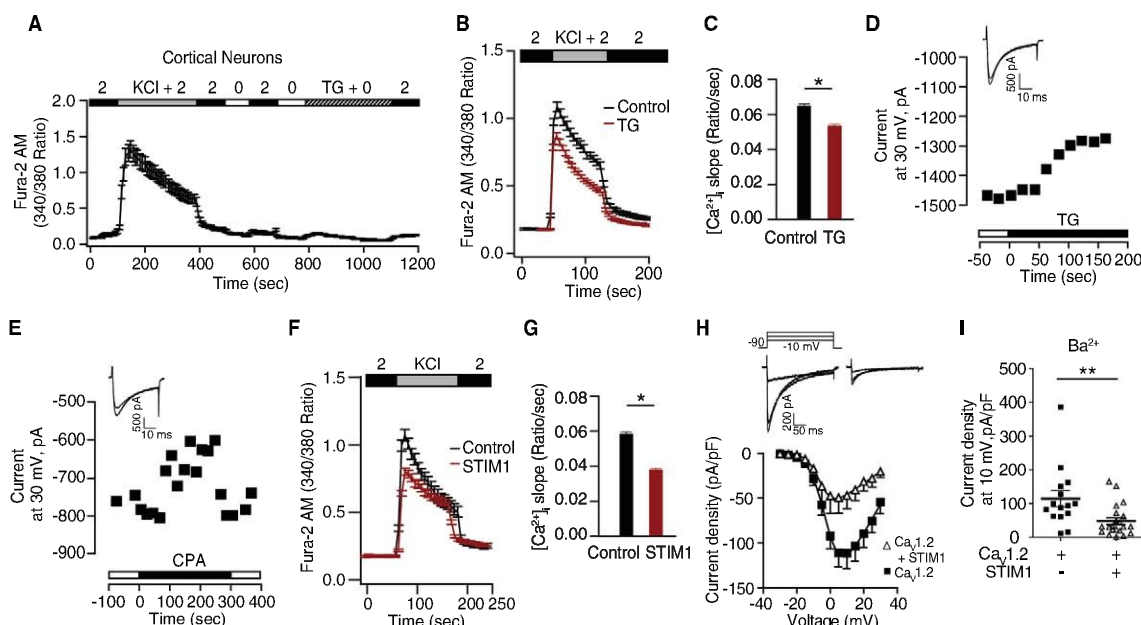


Fig. 1. Regulation of calcium influx through Ca_v1.2 by STIM1. **(A)** Fura-2 Ca²⁺ imaging of primary cortical neurons stimulated with KCl or with thapsigargin (TG; *n* = 17). Extracellular [Ca²⁺]_i is indicated. **(B and C)** Depolarization-induced increase in [Ca²⁺]_i in cortical neurons before and after treatment with TG **(B)** and measurements of the rate of increase in [Ca²⁺]_i (*n* = 18, **P* < 0.05) **(C)**. **(D and E)** Time course of Ca²⁺ currents measured in HEK293 cells expressing Ca_v1.2, before or after treatment with 1 μM TG or 5 μM CPA. **(F and**

G) [Ca²⁺]_i measurements of cortical neurons transfected with a control (*n* = 16) or a STIM1 plasmid (*n* = 18). Rate of change in [Ca²⁺]_i after depolarization for control and wild-type cells is shown in **(G)** (**P* < 0.05). **(H)** Current traces (above) and peak *I*-*V* relationships measured in HEK293 cells expressing Ca_v1.2 in the presence or absence of STIM1 with 5 mM Ba²⁺ as a charge carrier. **(I)** Normalized current amplitudes recorded in response to +10 mV depolarizing pulses.

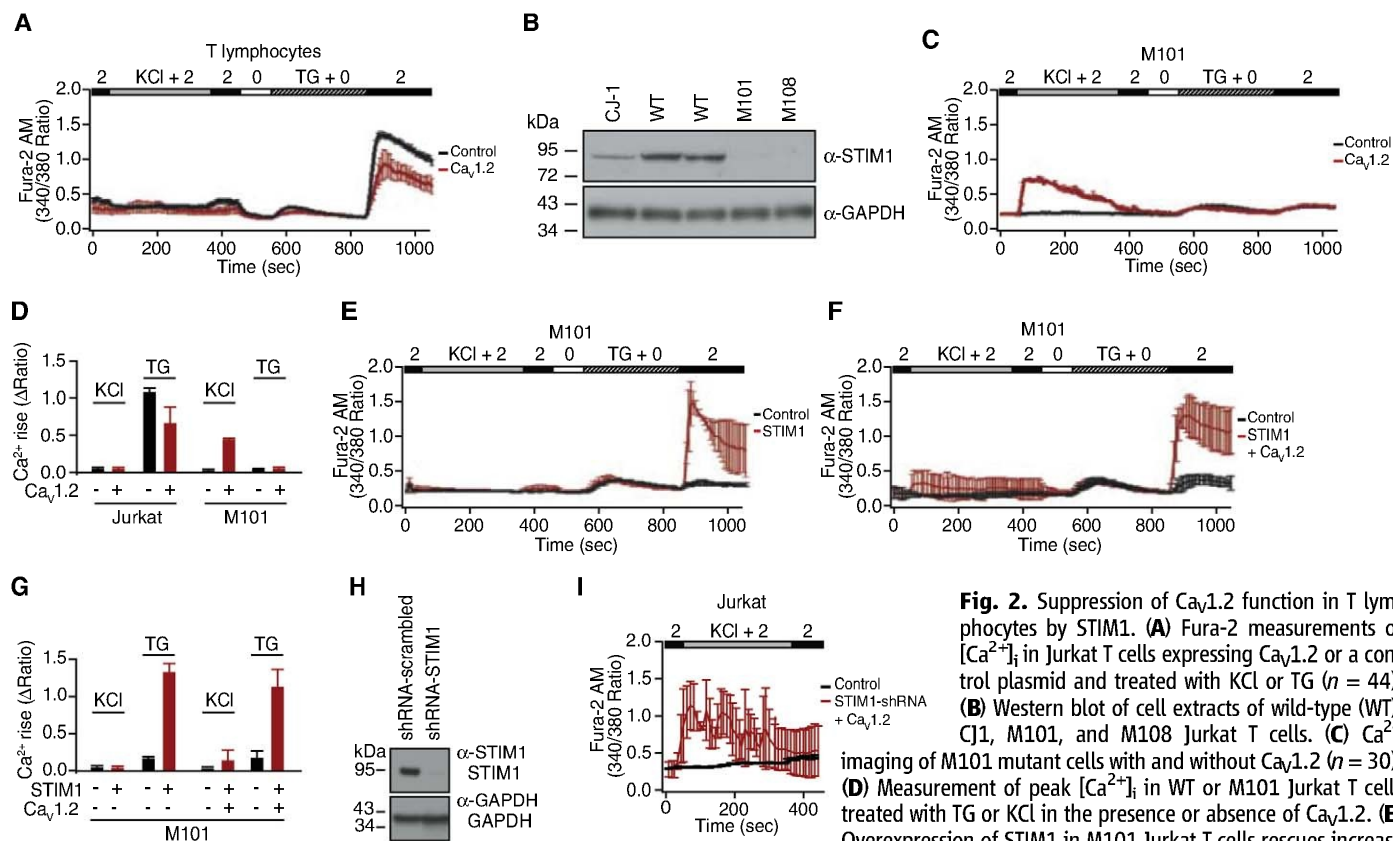


Fig. 2. Suppression of Ca_v1.2 function in T lymphocytes by STIM1. **(A)** Fura-2 measurements of [Ca²⁺]_i in Jurkat T cells expressing Ca_v1.2 or a control plasmid and treated with KCl or TG (*n* = 44). **(B)** Western blot of cell extracts of wild-type (WT), C1, M101, and M108 Jurkat T cells. **(C)** Ca²⁺ imaging of M101 mutant cells with and without Ca_v1.2 (*n* = 30). **(D)** Measurement of peak [Ca²⁺]_i in WT or M101 Jurkat T cells treated with TG or KCl in the presence or absence of Ca_v1.2. **(E)** Overexpression of STIM1 in M101 Jurkat T cells rescues increase in [Ca²⁺]_i induced by TG (*n* = 13). **(F)** Expression of STIM1 along with Ca_v1.2 reduces the KCl-induced [Ca²⁺]_i elevation in the M101 mutants (control, *n* = 15; STIM1/Ca_v1.2, *n* = 20). **(G)** Quantification of peak depolarization-induced [Ca²⁺]_i in M101 cells expressing STIM1 and Ca_v1.2 as indicated. **(H)** Western blot of extracts from cells expressing shRNA-STIM1 or shRNA-scrambled. **(I)** [Ca²⁺]_i measurements in WT Jurkat T cells expressing Ca_v1.2 in the presence or absence of the shRNA STIM1.

of $\text{Ca}_v1.2$ was reversible, we treated cells with the reversible sarco-endoplasmic reticulum calcium adenosine triphosphatase (SERCA) inhibitor cyclopiazonic acid (CPA) (Fig. 1E). Treatment of cells expressing $\text{Ca}_v1.2$ with CPA led to a 10% inhibition of $\text{Ca}_v1.2$ currents that was reversed after removal of CPA, which suggests that inhibition of $\text{Ca}_v1.2$ is not specific to TG and can be reversed by re-filling of stores (Fig. 1, D and E).

To determine whether STIM1 has a role in suppressing $\text{Ca}_v1.2$ currents after store depletion, we introduced STIM1 into cortical neurons and measured KCl-stimulated $[\text{Ca}^{2+}]_i$ elevations. Expression of STIM1 for 9 to 12 hours caused a decrease in the increase in $[\text{Ca}^{2+}]_i$ (Fig. 1F) that was also observed in Neuro2A neuroblastoma cells (fig. S1). To measure the effects of STIM1 on $\text{Ca}_v1.2$ currents directly, we measured Ba^{2+} (Fig. 1H) and Ca^{2+} (fig. S2) currents in HEK293 cells expressing $\text{Ca}_v1.2$ and STIM1. Expression of STIM1 eliminated $\text{Ca}_v1.2$ currents in about 40% of cells and reduced Ba^{2+} and Ca^{2+} currents in the rest, but had no effect on the I - V relationship of the channels (Fig. 1H). Thus, expression of STIM1 inhibits activation of both endogenous and heterologously expressed $\text{Ca}_v1.2$ channels.

In contrast to neurons, T lymphocytes show no voltage-dependent $[\text{Ca}^{2+}]_i$ elevations (10, 11) but have a large store-operated Ca^{2+} influx (Fig. 2A) pathway and high levels of STIM1. To test whether endogenous STIM1 can suppress $\text{Ca}_v1.2$ channels, we introduced yellow fluorescent protein (YFP)-labeled $\text{Ca}_v1.2$, along with $\beta 1b$ and $\alpha 2\delta 1$ subunits, into Jurkat T cells and measured the depolarization-induced Ca^{2+} influx (Fig. 2A). Although the YFP-labeled channel was clearly expressed, we did not detect any depolarization-induced increase in $[\text{Ca}^{2+}]_i$, consistent with the notion that STIM1 suppresses $\text{Ca}_v1.2$ (Fig. 2A). To determine whether STIM1 is necessary to suppress $\text{Ca}_v1.2$ activity in T cells, we used three lines of Jurkat T cells that lack store-operated Ca^{2+} influx (26). Sequencing the STIM1 and Orai genes in these cells revealed that three of them—CJ1, M101, and M108—contain either nonsense or missense mutations in the *stim1* gene (fig. S3 and table S1) and have significantly reduced amounts of STIM1 (Fig. 2B). We introduced $\text{Ca}_v1.2$, $\beta 1b$, and $\alpha 2\delta 1$ into all three cell lines and measured the depolarization-induced increase in $[\text{Ca}^{2+}]_i$. In contrast to wild-type Jurkat T cells, the three mutant cell lines showed voltage-activated

increases in $[\text{Ca}^{2+}]_i$, suggesting that loss of STIM1 allowed $\text{Ca}_v1.2$ expression in these cells (Fig. 2, C and D, and fig. S4). To confirm that this was due to loss of STIM1, we expressed STIM1 in the M101 cell line along with $\text{Ca}_v1.2$ (Fig. 2E). This restored store-operated Ca^{2+} entry in M101 cells and also prevented activation of $\text{Ca}_v1.2$ channels (Fig. 2, F and G). As a further test of the idea that STIM1 inhibits $\text{Ca}_v1.2$ channels in T cells, we designed a short hairpin RNA (shRNA) against STIM1. The STIM1 shRNA efficiently eliminated the expression of STIM1 protein relative to a scrambled shRNA control (Fig. 2H) and allowed the functional expression of $\text{Ca}_v1.2$ channels, indicating that STIM1 suppresses VGCC function in Jurkat cells (Fig. 2, H and I).

The observation that STIM1 suppresses $\text{Ca}_v1.2$ currents led us to test whether the two proteins interact in cells. We labeled $\text{Ca}_v1.2$ and STIM1 with FLAG and Myc epitope tags, respectively, and then expressed the proteins in HEK293T cells and measured their interaction by coimmunoprecipitation. Immunoprecipitation of FLAG-tagged $\text{Ca}_v1.2$ resulted in coimmunoprecipitation of Myc-tagged STIM1 only in cells expressing both proteins (Fig. 3A). The reverse experiment

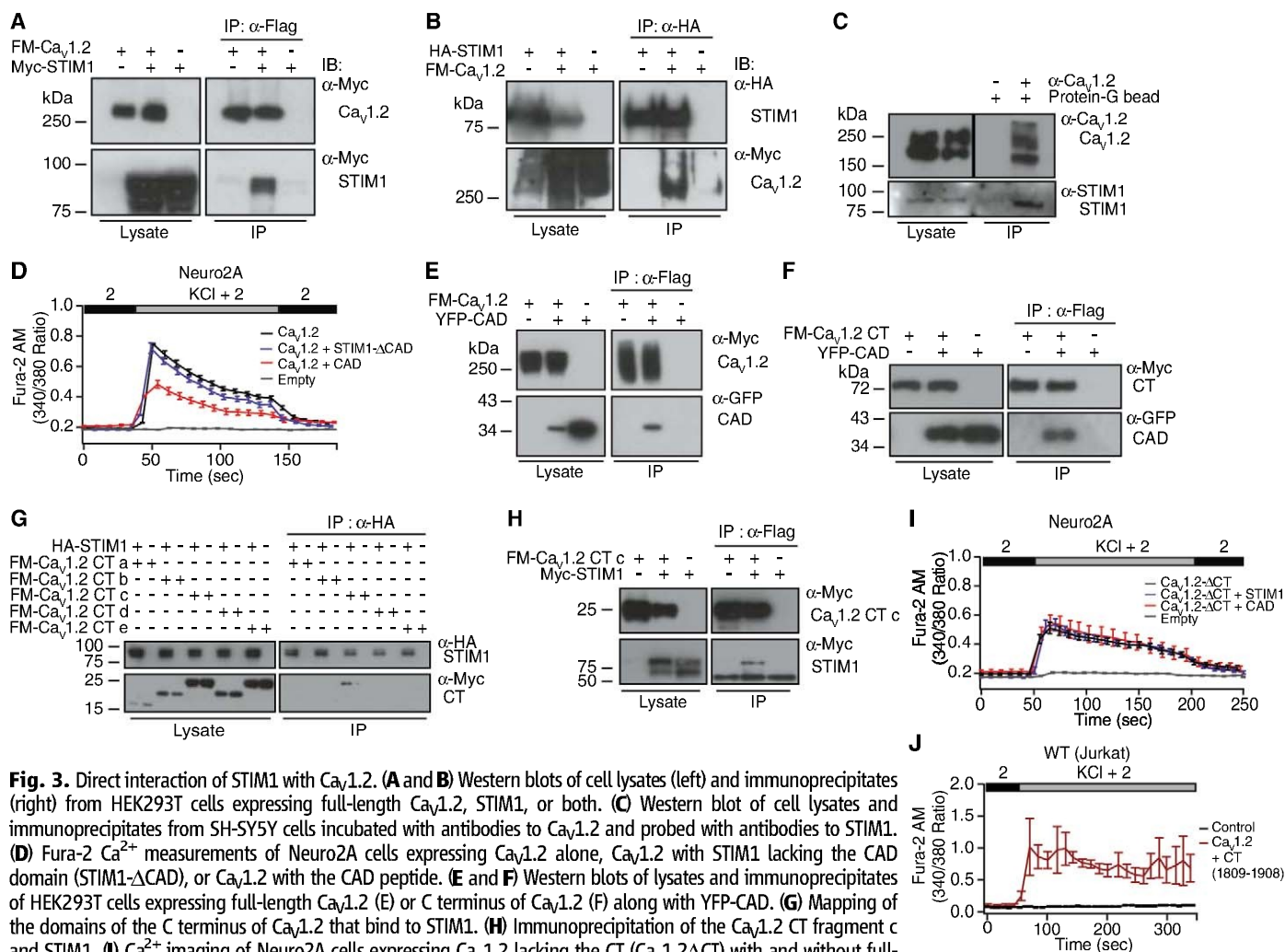


Fig. 3. Direct interaction of STIM1 with $\text{Ca}_v1.2$. (A and B) Western blots of cell lysates (left) and immunoprecipitates (right) from HEK293T cells expressing full-length $\text{Ca}_v1.2$, STIM1, or both. (C) Western blot of cell lysates and immunoprecipitates from SH-SY5Y cells incubated with antibodies to $\text{Ca}_v1.2$ and probed with antibodies to STIM1. (D) Fura-2 Ca^{2+} measurements of Neuro2A cells expressing $\text{Ca}_v1.2$ alone, $\text{Ca}_v1.2$ with STIM1 lacking the CAD domain (STIM1- Δ CAD), or $\text{Ca}_v1.2$ with the CAD peptide. (E and F) Western blots of lysates and immunoprecipitates of HEK293T cells expressing full-length $\text{Ca}_v1.2$ (E) or C terminus of $\text{Ca}_v1.2$ (F) along with YFP-CAD. (G) Mapping of the domains of the C terminus of $\text{Ca}_v1.2$ that bind to STIM1. (H) Immunoprecipitation of the $\text{Ca}_v1.2$ CT fragment c and STIM1. (I) Ca^{2+} imaging of Neuro2A cells expressing $\text{Ca}_v1.2$ lacking the CT ($\text{Ca}_v1.2\Delta\text{CT}$) with and without full-length STIM1 or the CAD domain. (J) Ca^{2+} imaging of Jurkat T cells expressing $\text{Ca}_v1.2$ alone or along with $\text{Ca}_v1.2$ -CT fragment c peptide ($n = 10$).

of immunoprecipitating STIM1 and blotting for $\text{Ca}_v1.2$ confirmed that the two proteins form a complex when they are expressed in cells (Fig. 3B). To determine whether the two proteins also interact when expressed at endogenous levels, we performed coimmunoprecipitation of $\text{Ca}_v1.2$ and STIM1 from SH-SY5Y human neuroblastoma cells that express both proteins. STIM1 was associated with immunoprecipitated $\text{Ca}_v1.2$, indicating that the interaction also occurs under endogenous conditions (Fig. 3C).

The CAD of STIM1 mediates the binding and activation of Orai1 channels. We therefore investigated whether this domain is necessary for inhibiting $\text{Ca}_v1.2$ (24). We introduced STIM1 lacking the CAD (STIM1 Δ CAD) or the CAD peptide alone into cells expressing $\text{Ca}_v1.2$. STIM1 Δ CAD did not suppress depolarization-induced $[\text{Ca}^{2+}]_i$ increase (Fig. 3D), whereas CAD alone caused 50% suppression of the $[\text{Ca}^{2+}]_i$ increase, indicating that this domain is necessary and sufficient for inhibition of $\text{Ca}_v1.2$ (Fig. 3D). To determine whether the CAD binds to $\text{Ca}_v1.2$, we introduced YFP-tagged CAD into HEK293T cells expressing $\text{Ca}_v1.2$. Immunoprecipitation of the CAD peptide resulted in coimmunoprecipitation of the channel, indicating that the CAD can bind to $\text{Ca}_v1.2$ independently of the rest of STIM1 (Fig. 3E).

To find which domains of $\text{Ca}_v1.2$ bind to the STIM1 CAD, we overexpressed the CAD along with the intracellular loops of $\text{Ca}_v1.2$ in HEK293T

cells and investigated their binding by coimmunoprecipitation. Immunoprecipitation of the CAD peptide resulted in strong coimmunoprecipitation of the C terminus of $\text{Ca}_v1.2$ (Fig. 3F). We subdivided the C-terminal domain into five peptides and tested which domain coimmunoprecipitated with the STIM1 CAD. A region between amino acids 1809 and 1908 of the C terminus of $\text{Ca}_v1.2$ interacted with the CAD of STIM1 (Fig. 3G). This same domain also interacted with full-length STIM1 protein, indicating that this region can interact with the CAD in its endogenous context (Fig. 3H).

If interaction between $\text{Ca}_v1.2$ and STIM1 is required to inhibit channel conductance, then a channel lacking the C-terminal domain should not be suppressed by STIM1. We expressed $\text{Ca}_v1.2$ lacking the C terminus ($\text{Ca}_v1.2\Delta\text{CT}$) along with STIM1 in mouse Neuro2A cells. Introduction of the $\text{Ca}_v1.2\Delta\text{CT}$ led to a depolarization-induced $[\text{Ca}^{2+}]_i$ rise in these cells that was unaffected by coexpression of STIM1 or the STIM1 CAD (Fig. 3I). Thus, binding of STIM1 to the $\text{Ca}_v1.2$ C terminus is required for suppression of channel function. To test this further, we expressed the $\text{Ca}_v1.2$ C terminus in Jurkat T cells to determine whether this domain would bind to endogenous STIM1 and prevent it from suppressing $\text{Ca}_v1.2$ channels. In contrast to cells expressing $\text{Ca}_v1.2$ alone, which showed no increase in $[\text{Ca}^{2+}]_i$ after depolarization, cells expressing the channels and the peptide showed a large increase in $[\text{Ca}^{2+}]_i$ (Fig. 3J), indicating that the C terminus of $\text{Ca}_v1.2$

is critical for the ability of STIM1 to suppress the activity of $\text{Ca}_v1.2$ channels.

We next investigated whether STIM1 and $\text{Ca}_v1.2$ colocalize in cells after depletion of stores. We introduced hemagglutinin (HA)-tagged $\text{Ca}_v1.2$ and FLAG-tagged STIM1 into HEK293 cells and used quantitative immunocytochemistry to examine their colocalization. We observed significant colocalization of the two proteins in resting cells and a slight increase after treatment with 1 μM TG (Fig. 4A). Because $\text{Ca}_v1.2$ and STIM1 interacted significantly before store depletion and seemed to colocalize in intracellular vesicles, we examined whether STIM1 altered the surface expression of $\text{Ca}_v1.2$ channels. We transfected hippocampal neurons with $\text{Ca}_v1.2$ containing an intracellular YFP and an extracellular HA tag (27) and measured the surface fraction of $\text{Ca}_v1.2$ by staining unpermeabilized cells with fluorescent antibodies to HA (Fig. 4, B to D). In the absence of STIM1, a large fraction of the $\text{Ca}_v1.2$ channels were expressed on the cell surface, but introduction of STIM1 led to a 73% decline in cell surface $\text{Ca}_v1.2$ channels. To determine whether STIM1 increases the internalization of $\text{Ca}_v1.2$, we expressed dominant negative dynamin-1 (DN-dyn1), a potent inhibitor of internalization, in cells expressing $\text{Ca}_v1.2$ and STIM1 (Fig. 4, B to D). DN-dyn1 prevented the loss of $\text{Ca}_v1.2$ cell surface expression in cells expressing STIM1, providing evidence that STIM1 causes internalization of the channels.

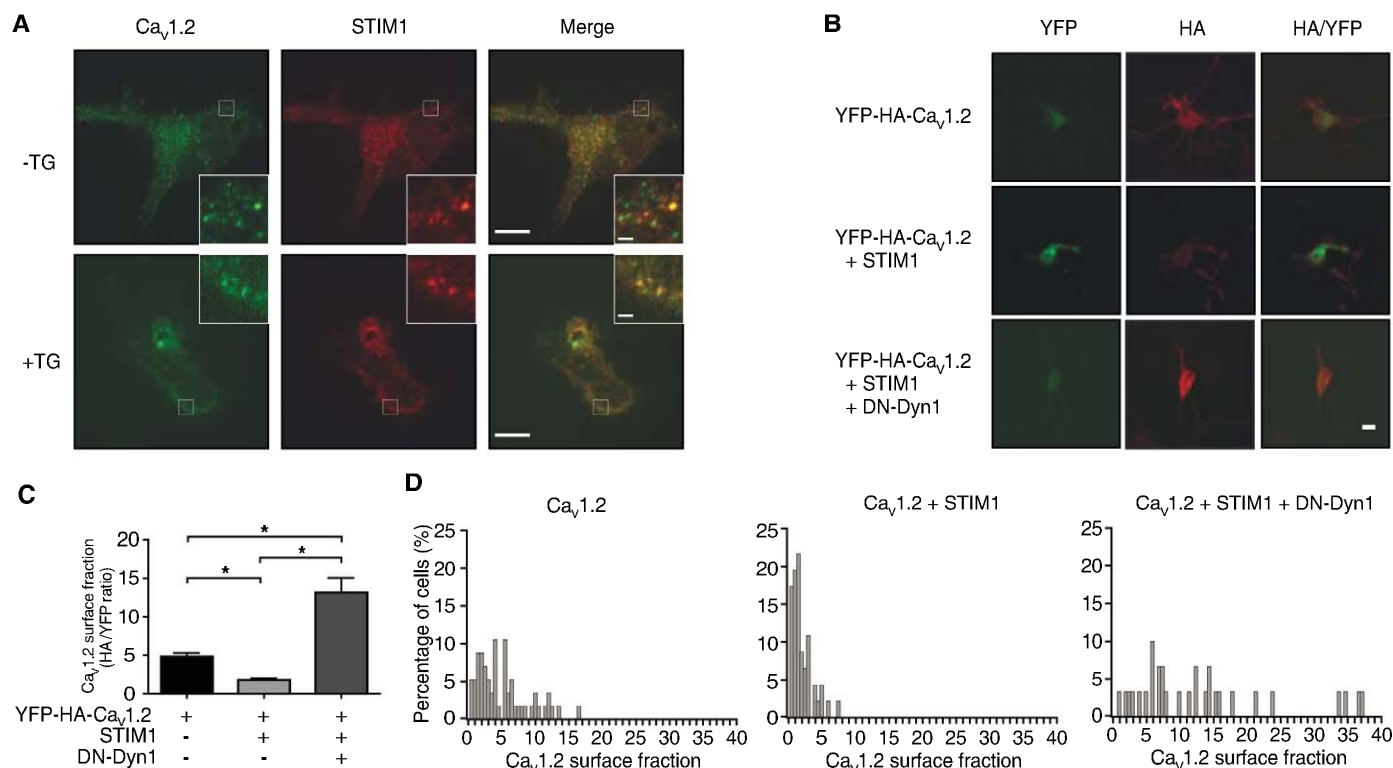


Fig. 4. Regulation of surface expression of $\text{Ca}_v1.2$ by STIM1. **(A)** Hippocampal neurons expressing HA- $\text{Ca}_v1.2$ and FLAG-STIM1 were fixed and stained with antibodies to HA or FLAG. Cells are shown before and after treatment with 1 μM TG. **(B)** YFP and HA staining of hippocampal neurons expressing $\text{Ca}_v1.2$

containing an N-terminal YFP and an extracellular HA tag along with STIM1, or STIM1 and dominant negative dynamin 1 (DN-Dyn1). **(C)** Measurement of the fraction of $\text{Ca}_v1.2$ channels on the cell surface from the experiment shown in **(B)** ($n > 50$; mean $\pm$ SEM). **(D)** Histogram of $\text{Ca}_v1.2$ cell surface expression.

Our studies support a model in which STIM1 is recruited to $\text{Ca}_v1.2$ channels after depletion of stores. It acutely reduces $\text{Ca}_v1.2$ currents by about 15% and chronically triggers $\text{Ca}_v1.2$ internalization, leading to complete loss of functional $\text{Ca}_v1.2$ channels. STIM1 therefore acts as switch that promotes Ca^{2+} entry through Orai channels and inhibits Ca^{2+} entry through $\text{Ca}_v1.2$. This is likely to be important for selecting among different Ca^{2+} -activated signaling cascades, as Orai and $\text{Ca}_v1.2$ activate different signaling pathways. In addition, regulation of $\text{Ca}_v1.2$ by STIM1 could be important for the inhibition of $\text{Ca}_v1.2$ that occurs after activation of PLC-coupled receptors such as the muscarinic acetylcholine receptor and the D2 dopamine receptor (28, 29). Together these findings provide a previously unknown set of functions for STIM1 in mammalian cells.

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Supporting Online Material

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Materials and Methods
Figs. S1 to S4
Table S1

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The Calcium Store Sensor, STIM1, Reciprocally Controls Orai and $\text{Ca}_v1.2$ Channels

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Calcium signals, pivotal in controlling cell function, can be generated by calcium entry channels activated by plasma membrane depolarization or depletion of internal calcium stores. We reveal a regulatory link between these two channel subtypes mediated by the ubiquitous calcium-sensing STIM proteins. STIM1 activation by store depletion or mutational modification strongly suppresses voltage-operated calcium ($\text{Ca}_v1.2$) channels while activating store-operated Orai channels. Both actions are mediated by the short STIM-Orai activating region (SOAR) of STIM1. STIM1 interacts with $\text{Ca}_v1.2$ channels and localizes within discrete endoplasmic reticulum/plasma membrane junctions containing both $\text{Ca}_v1.2$ and Orai1 channels. Hence, STIM1 interacts with and reciprocally controls two major calcium channels hitherto thought to operate independently. Such coordinated control of the widely expressed $\text{Ca}_v1.2$ and Orai channels has major implications for Ca^{2+} signal generation in excitable and nonexcitable cells.

C a^{2+} entry channels, crucial in providing cellular Ca^{2+} signals, are controlled by sensing mechanisms, including membrane voltage, surface receptors, and Ca^{2+} -sensing STIM proteins in the endoplasmic reticulum (ER) (1). The coordinated operation of these transduction processes is key to controlling cell function. STIM proteins are dynamic ER Ca^{2+} sensors that ag-

gregate when ER is depleted of Ca^{2+} , then rapidly translocate into ER-plasma membrane (PM) junctions, where they interact with and activate the highly Ca^{2+} -selective Orai family of PM channels (2, 3). We determined that STIM proteins also mediate inhibitory control of voltage-activated $\text{Ca}_v1.2$ channels. This action was independent of Orai channel function or changes in cytosolic Ca^{2+} and was mediated by a direct action of STIM1 on the $\text{Ca}_v1.2$ α_1C subunit. Thus, STIM1 reciprocally controls Orai and $\text{Ca}_v1.2$ channels, indicating a hitherto unknown and potentially crucial regulatory link between receptor-induced Ca^{2+} store depletion and control of voltage-activated Ca^{2+} signals.

We studied STIM1-mediated Ca^{2+} entry signals in A7r5 clonal vascular smooth muscle cells

(VSMC). Ca^{2+} store depletion by vasopressin (VP) or ER Ca^{2+} pump blockade by thapsigargin (TG) induced a large Ca^{2+} influx across the PM when Ca^{2+} was added externally (Fig. 1, A and B). However, the inwardly rectifying Ca^{2+} -release activated Ca^{2+} (CRAC) current mediating this Ca^{2+} entry was barely measurable (Fig. 1B, inset) because only a small number of Ca^{2+} ions flow through these highly selective channels. Expression in A7r5 cells of the STIM1-D76A mutant defective in sensing ER Ca^{2+} (I) selectively activated Ca^{2+} entry (almost no entry of Sr^{2+}) (Fig. 1C), which required no store emptying. Expression of the E106A Orai1 mutant lacking a functional pore and acting as a dominant negative on Orai channels (4, 5) completely eliminated VP- or TG-induced Ca^{2+} entry (Fig. 1A and B), revealing that entry is exclusively Orai-mediated.

Canonical transient receptor potential (TRPC) channels, widely reported to contribute to store-induced Ca^{2+} entry (I, 6), are highly expressed in VSMCs (7). In A7r5 cells, a large nonselective current activated by VP and mediated by TRPCs (7) was not influenced by store depletion or by expression of the dominant Orai1-E106A mutant or the constitutively active STIM1-D76A mutant, reinforcing recent evidence (8) against a role of STIM proteins or Ca^{2+} stores in TRPC channel function. In contrast, endogenous $\text{Ca}_v1.2$ channels did respond to emptying of intracellular Ca^{2+} stores. In A7r5 cells, Sr^{2+} entry through $\text{Ca}_v1.2$ channels was activated by a depolarizing pulse of high extracellular $[\text{K}^+]$ and blocked by the $\text{Ca}_v1.2$ -specific blocker nimodipine (Fig. 1D). If 2 μM ionomycin or 2 μM TG was added to deplete Ca^{2+} stores, a subsequent depolarizing pulse resulted in greatly decreased $\text{Ca}_v1.2$ -mediated entry of Sr^{2+} (Fig. 1, E and G). However, although VP treatment also induced Ca^{2+} store depletion, $\text{Ca}_v1.2$ -mediated Sr^{2+} entry was instead enhanced (Fig.

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1, F and G), likely due to activation of $\text{Ca}_v1.2$ channels by Ca^{2+} - and diacylglycerol-induced protein kinase C stimulation (9, 10). To more directly examine the actions of store emptying on

$\text{Ca}_v1.2$ channels, we measured $\text{Ca}_v1.2$ currents by patch-clamping cells with cytosolic Ca^{2+} buffered at physiological resting levels with the Ca^{2+} chelator EGTA to prevent any Ca^{2+} -dependent

changes in $\text{Ca}_v1.2$ function. Compared with control $\text{Ca}_v1.2$ peak current (0.28 ± 0.08 pA/pF), store depletion by ionomycin inhibited $\text{Ca}_v1.2$ current by 96% (0.01 ± 0.02 pA/pF; $n = 6$, $P =$

Fig. 1. Store-dependent control of Orai and $\text{Ca}_v1.2$ channels. Fura-2 F_{340}/F_{380} ratiometric responses to cytosolic Ca^{2+} in A7r5 VSMCs in response to 100 nM VP (A) or 2 μM TG (B), in control or Orai1-E106A-CFP-transfected cells in nominally Ca^{2+} free or 3 mM external Ca^{2+} (bars). (Inset) Current-voltage curve for CRAC channels in control A7r5 cells. (C) Fura-2 responses in control and STIM1-D76A-transfected A7r5 cells (bars, 3 mM external Sr^{2+} or Ca^{2+}). (D) Fura-2 responses to application of external 134 mM K^{+} with 3 mM Sr^{2+} (bars, $\text{K}^{+}/\text{Sr}^{2+}$); 2 μM nimodipine (arrow). (E) Fura-2 responses to two sequential $\text{K}^{+}/\text{Sr}^{2+}$ pulses (bars) with addition of dimethyl sulfoxide (DMSO) or 2 μM ionomycin (arrow), and subsequent 2 μM nimodipine addition. (F) As in (E), except with the addition of 100 nM VP. (G) Statistics for results in (E), (F), and TG (trace not shown) ($n = 6$, paired t test). (H) IV curve for whole-cell Ba^{2+} current through $\text{Ca}_v1.2$ channels in A7r5 cells with cytosolic Ca^{2+} clamped to 0.1 μM (10 mM EGTA; 3.4 mM CaCl_2), either before (left) or 5 min after store depletion with 2 μM ionomycin, 100 nM VP, or control (right). (I to K) Fura-2 responses to $\text{K}^{+}/\text{Sr}^{2+}$ in HEK293 cells expressing three $\text{Ca}_v1.2$ subunits ($\alpha_{1C} + \beta_{2a} + \alpha_{2\delta 1}$) or control-transfected cells. (I) $\text{K}^{+}/\text{Sr}^{2+}$ responses in $\text{Ca}_v1.2$ -transfected and control-transfected cells; 2 μM nimodipine (arrow); inset, IV curve for $\text{Ca}_v1.2$ -transfected cells ($n = 4$). (J) Fura-2 responses to sequential $\text{K}^{+}/\text{Sr}^{2+}$ pulses (bars) with additions of DMSO or 2 μM ionomycin (arrow), and 2 μM nimodipine (arrows). (K) $\text{K}^{+}/\text{Sr}^{2+}$ responses after DBHQ (10 μM , 10 min, present throughout) or TPEN (250 μM , 5 min, removed at $\text{K}^{+}/\text{Sr}^{2+}$ addition).

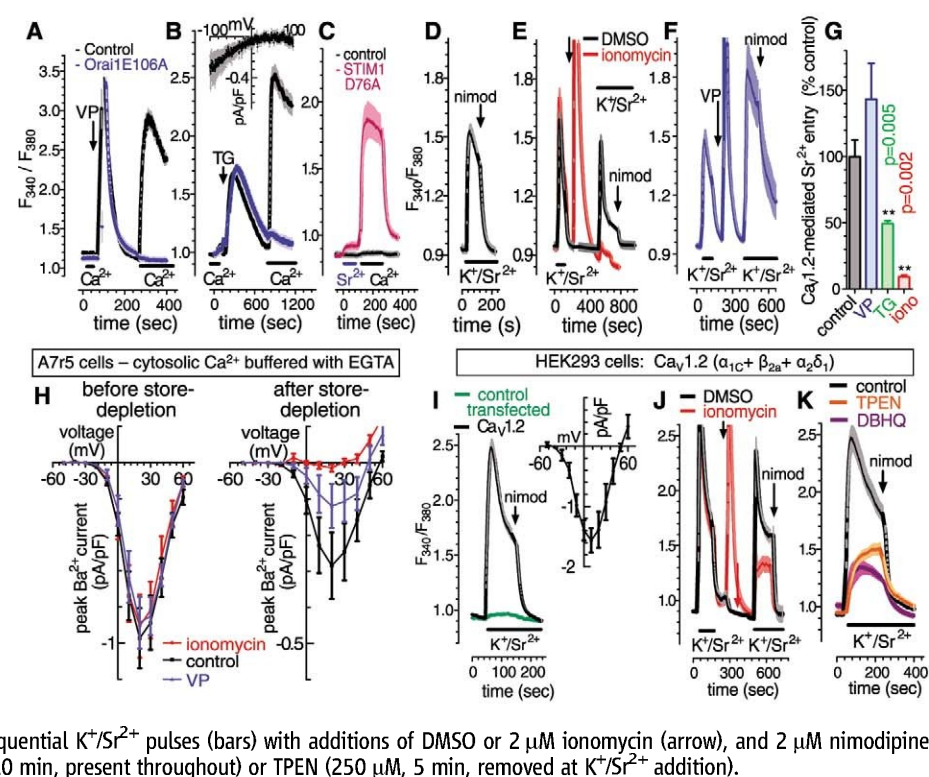
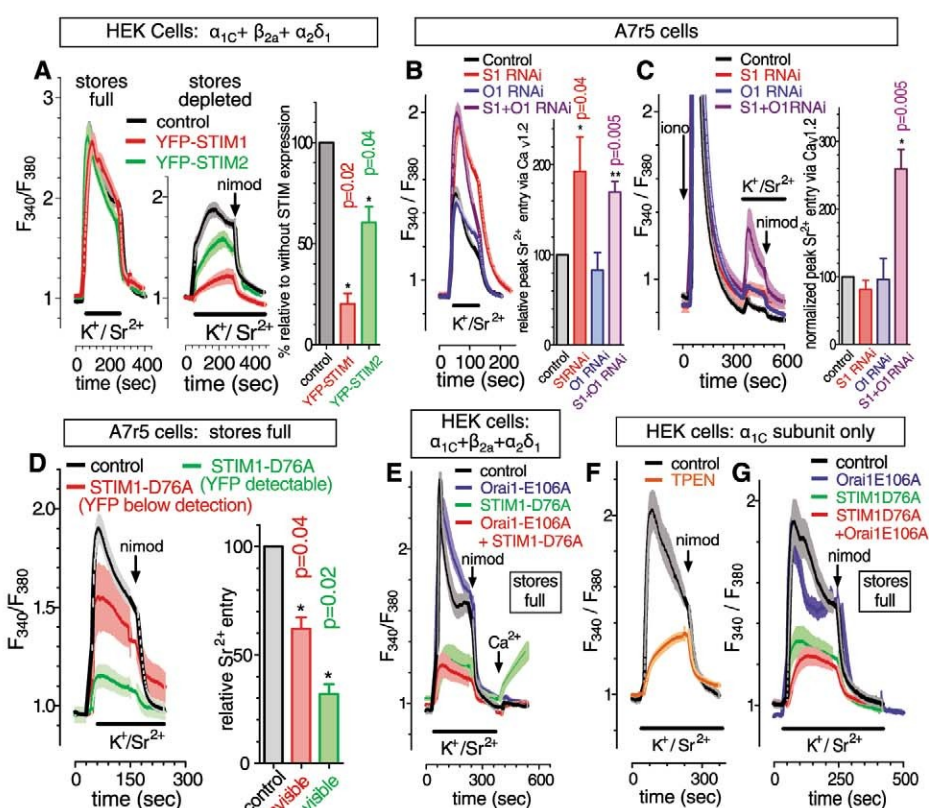


Fig. 2. STIM proteins mediate store depletion-induced control of $\text{Ca}_v1.2$ channels. (A) HEK293 cells expressing $\alpha_{1C} + \beta_{2a} + \alpha_{2\delta 1}$ $\text{Ca}_v1.2$ channel subunits; fura-2 responses to $\text{K}^{+}/\text{Sr}^{2+}$ pulses (bars) before (left) and after (middle) store emptying with 2 μM ionomycin and subsequent 2 μM nimodipine addition (arrow). Statistics (right), paired t test, $n = 6$. (B) Store-replete A7r5 cells treated either with STIM1 RNAi, Orai1 RNAi, STIM1+Orai1 RNAi, or control RNAi; left, fura-2 responses to $\text{K}^{+}/\text{Sr}^{2+}$ pulse (bar); right, statistics (paired t test, $n = 8$). (C) The same RNAi-treated cells used in (B) were then store-depleted with 2 μM ionomycin (arrow); left, fura-2 responses to $\text{K}^{+}/\text{Sr}^{2+}$ pulse (bar); right, statistics (paired t test, $n = 8$). (D) Store-replete A7r5 cells either control-transfected or transfected with YFP-STIM1-D76A (cells with detectable or undetectable YFP on a single coverslip); left, fura-2 responses to $\text{K}^{+}/\text{Sr}^{2+}$ pulse (bar) and 2 μM nimodipine (arrow); right, statistics (paired t test, $n = 8$). (E) HEK293 cells expressing $\alpha_{1C} + \beta_{2a} + \alpha_{2\delta 1}$ $\text{Ca}_v1.2$ channel subunits cotransfected with either Orai1-E106A-CFP, YFP-STIM1-D76A, Orai1-E106A-CFP + YFP-STIM1-D76A, or control plasmid; fura-2 responses to $\text{K}^{+}/\text{Sr}^{2+}$ pulse (bar), 2 μM nimodipine (arrow) and 3 mM Ca^{2+} (arrow). (F) HEK293 cells expressing α_{1C} alone; fura-2 response to $\text{K}^{+}/\text{Sr}^{2+}$ pulse (bar) and 2 μM nimodipine (arrow) in cells either TPEN-treated (250 μM , 5 min, removed at $\text{K}^{+}/\text{Sr}^{2+}$ addition) or untreated. (G) HEK293 cells expressing α_{1C} cotransfected with either Orai1-E106A-CFP, YFP-STIM1-D76A, Orai1-E106A-CFP + YFP-STIM1-D76A, or control plasmid; fura-2 response to $\text{K}^{+}/\text{Sr}^{2+}$ pulse (bar), and 2 μM nimodipine (arrow).



0.001) (Fig. 1H). Store depletion with VP also reduced current by 58% (0.11 ± 0.06 pA/pF; $n = 6$, $P = 0.004$). Thus, Ca^{2+} buffering revealed a consistent store depletion-mediated inhibition of $\text{Ca}_v1.2$ channels. We further examined the influence of Ca^{2+} store depletion on $\text{Ca}_v1.2$ channels expressed in human embryonic kidney (HEK293) cells. Nimodipine-sensitive, depolarization-induced Sr^{2+} entry (Fig. 1I) with typical $\text{Ca}_v1.2$ current-voltage relationship (inset) was observed in HEK293 cells expressing the three $\text{Ca}_v1.2$ component subunits α_{1C} , β_{2a} , and $\alpha_{2\delta 1}$. Ca^{2+} store depletion with ionomycin or ER Ca^{2+} pump blocker di-tert-butylhydroquinone (DBHQ) substantially reduced $\text{Ca}_v1.2$ -mediated Sr^{2+} entry compared with control cells (Fig. 1, J and K). Application of the cell-permeant intraluminal ER- Ca^{2+} chelator, N,N,N',N' -tetrakis(2-pyridylmethyl)-ethylenediamine (TPEN), caused a similar decrease (Fig. 1K). TPEN reduces ER- Ca^{2+} with no increase in cytosolic Ca^{2+} (11), confirming that store depletion inhibits $\text{Ca}_v1.2$ channels independent of cytosolic Ca^{2+} changes.

The ER Ca^{2+} sensors, STIM1 and STIM2, are triggered by ER Ca^{2+} depletion to translocate and activate PM Orai channels (1). We investigated the role of STIM proteins in store depletion-induced inhibition of $\text{Ca}_v1.2$ channels. Using $\text{Ca}_v1.2$ ($\alpha_{1C} + \beta_{2a} + \alpha_{2\delta 1}$)-transfected HEK293 cells, overexpressed yellow fluorescent protein-fusion constructs (YFP-STIM1 or YFP-STIM2) (fig. S1, A to D) had little effect on $\text{Ca}_v1.2$ -mediated Sr^{2+} entry in store-replete cells (Fig. 2A). However, YFP-STIM1 expression greatly increased the inhibitory effect of ionomycin-induced store depletion on $\text{Ca}_v1.2$ -mediated Sr^{2+} entry (Fig. 2A). Similar expression of YFP-STIM2 had a smaller effect. STIM2 is similarly poorer in coupling to activate Orai channels (1, 12, 13). Although native STIM1 is expressed in both ER and PM, YFP-STIM1 is exclusively expressed in ER (1, 14); thus, the action of STIM1 on $\text{Ca}_v1.2$ function is mediated by ER-STIM1.

We examined how STIM1 and Orai1 RNA interference (RNAi) influenced endogenous $\text{Ca}_v1.2$ channels in A7r5 cells. STIM1-siRNA (small in-

terfering RNA) increased $\text{Ca}_v1.2$ -mediated Sr^{2+} entry in store-replete cells (Fig. 2B), suggesting that some endogenous STIM1 exists in junctions and constitutively inhibits $\text{Ca}_v1.2$. Orai1-siRNA had little effect. STIM1-siRNA had little effect on store depletion-induced inhibition of $\text{Ca}_v1.2$ channels (Fig. 2C). However, although RNAi reduced STIM1 expression by ~80% (fig. S2A), store emptying may efficiently cause the remaining 20% to enter ER-PM junctions, perhaps explaining the differential effectiveness of STIM1-RNAi on $\text{Ca}_v1.2$ activity in store-replete versus store-depleted cells. A combination of STIM1- and Orai1-siRNA resulted in a substantial reduction in the inhibition of $\text{Ca}_v1.2$ channels by store depletion (Fig. 2C). After store depletion, Orai1 traps STIM1 within ER-PM junctions (15); thus, with limited STIM1 in RNAi-treated cells, endogenous Orai1 may be necessary to bring STIM1 to the PM where it can alter $\text{Ca}_v1.2$ channels.

STIM1 EF-hand mutants fail to bind Ca^{2+} (16–18), aggregate and translocate into ER-PM junctions, and constitutively activate Orai1 chan-

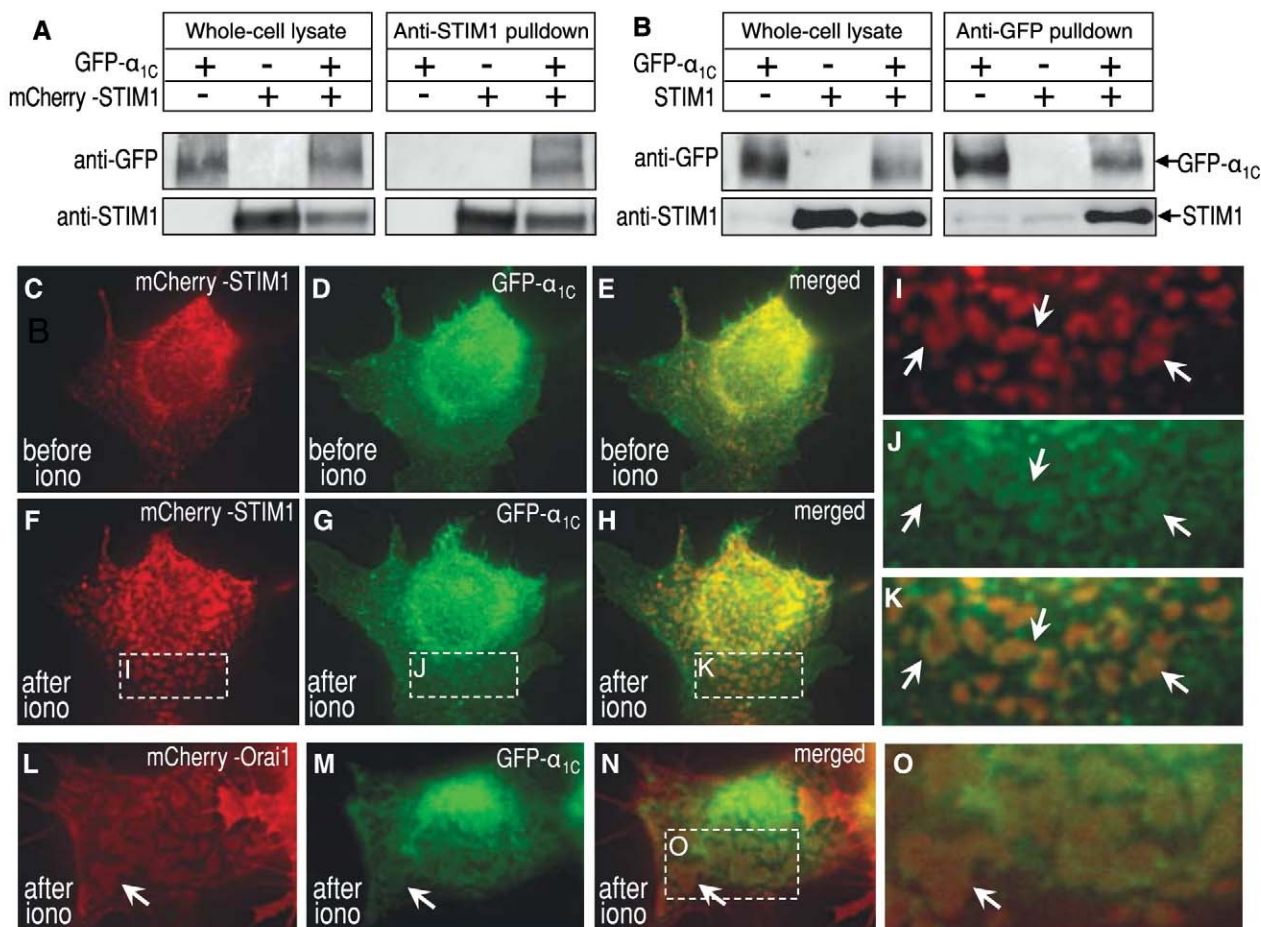


Fig. 3. Interactions between STIM1 and $\text{Ca}_v1.2$ α_{1C} , and store-dependent colocalization of STIM1, $\text{Ca}_v1.2$, and Orai1 proteins. (A) HEK293 cells coexpressing mCherry-STIM1 and GFP- α_{1C} ; whole-cell lysates (left) and antibody to STIM1 immunoprecipitates were probed with antibody to GFP and antibody to STIM1. (B) HEK293 cells coexpressing GFP- α_{1C} and STIM1; whole-cell lysates (left) and antibody to GFP immunoprecipitates were probed with antibody to GFP and antibody to STIM1. (C to O) High-resolution imaging focused on the ER-PM

interface in HEK293 cells. (C to H) Distribution of expressed mCherry-STIM1 and GFP- α_{1C} (coexpressed with $\beta_{2a} + \alpha_{2\delta 1}$ subunits), examined in cells either just before (C and E) or 5 min after (F to H) Ca^{2+} store depletion with 2 μM ionomycin. (I to K) Magnification of the STIM1, α_{1C} , and merged images, respectively. (L and M) Distribution of coexpressed mCherry-Orai1 and GFP- α_{1C} (with $\beta_{2a} + \alpha_{2\delta 1}$ subunits) in cells 5 min after ionomycin-induced emptying. (O) Magnified area of the merged image from (N).

nels without requiring changes in ER Ca^{2+} (18). Expression of YFP-STIM1-D76A EF-hand mutant in A7r5 cells led to a constitutive reduction in the function of endogenous $\text{Ca}_v1.2$ channels (Fig. 2D). Cells with visible YFP expression had >70% reduced $\text{Ca}_v1.2$ -mediated Sr^{2+} entry, and almost all mutant STIM1 was within clearly discernible junctions (fig. S2B). There was little difference in resting cytosolic $[\text{Ca}^{2+}]$ in D76A-expressing cells (fig. S2C), consistent with studies on cells stably expressing D76A (19). Cells with no detectable fluorescence had 40% reduced $\text{Ca}_v1.2$ function (Fig. 2D) and no change in resting Ca^{2+} (fig. S2C). Hence, the action of STIM1 on $\text{Ca}_v1.2$ channel function is independent of both luminal and cytosolic Ca^{2+} changes.

The same inhibitory effect of STIM1-D76A was observed on Sr^{2+} entry through $\text{Ca}_v1.2$ channels expressed in HEK cells (Fig. 2E). Coexpression of the dominant negative Orai1-E106A mutant did not alter the STIM1-D76A-induced inhibition of $\text{Ca}_v1.2$ channels, although it completely blocked the constitutive Ca^{2+} entry observed after addition of Ca^{2+} in STIM1-D76A-expressing cells after washout of the $\text{K}^+/\text{Sr}^{2+}$ solution (Fig. 2E, red versus green traces). Hence, we conclude that the inhibitory effect of STIM1 on $\text{Ca}_v1.2$ channels does not require Orai channel function and does not reflect any passage of ions through store-operated channels.

$\text{Ca}_v1.2$ channels comprise three subunits: the α_{1C} pore-forming moiety and the β and $\alpha_2\delta_1$ auxiliary subunits (10). STIM1-mediated $\text{Ca}_v1.2$ inhibition did not require the auxiliary proteins. As for HEK293 cells expressing all three $\text{Ca}_v1.2$ subunits, cells expressing only the α_{1C} subunit had depolarization-induced Sr^{2+} entry inhibited by

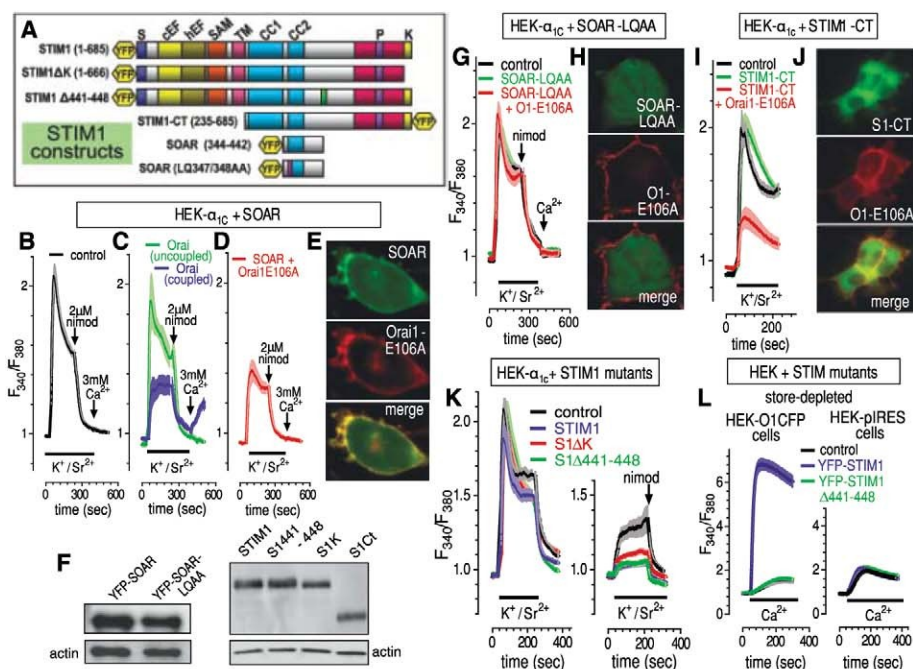
TPEN (Fig. 2F), enhanced with STIM1 or STIM2 overexpression (fig. S3, A to C) and mimicked by STIM1-D76A expression independently of Orai function (Fig. 2G). STIM1-D76A did not alter α_{1C} expression or vice versa, and α_{1C} did not alter STIM1-D76A localization (fig. S3, D and E).

To address whether $\text{Ca}_v1.2$ α_{1C} interacts with STIM1, we undertook coimmunoprecipitation studies using green fluorescent protein (GFP)-tagged α_{1C} and either native or mCherry-tagged STIM1. Coexpressed in HEK cells, antibody to STIM1 precipitates contained GFP- α_{1C} (Fig. 3A). Moreover, precipitates of GFP- α_{1C} with antibody to GFP revealed bound STIM1 (Fig. 3B). Although interactions between the two proteins exist, store depletion did not detectably alter this association. We undertook high-resolution imaging studies to examine distribution of STIM1, $\text{Ca}_v1.2$ channels, and Orai channels at the PM (19). We expressed N-tagged GFP- α_{1C} (20), β_{2a} and $\alpha_2\delta_1$ subunits, and N-tagged mCherry-STIM1 (19). High-resolution imaging of the ER-PM junctions adjacent to the coverslip revealed precise colocalization between mCherry-STIM1 and GFP- α_{1C} induced by emptying Ca^{2+} stores with ionomycin (Fig. 3, C to K). Ionomycin-mediated store depletion for 5 min induced mCherry-STIM1 to move into clearly defined ER-PM junctional areas (Fig. 3, C and F). GFP- $\text{Ca}_v1.2$ moved into the same areas (Fig. 3, D, G, and H). High magnification of the boxed areas (Fig. 3, I to K) reveals the precise nature of the colocalization of the two proteins after store depletion. There were no areas of STIM1 not colocalized with α_{1C} , although there were areas of α_{1C} extending beyond the STIM1-defined junctional areas. Small areas with colocalized STIM1- $\text{Ca}_v1.2$ were evident in

store-replete cells (Fig. 3, C to E). This indicates a possible constitutive role of STIM1 in attenuating $\text{Ca}_v1.2$ activity, consistent with the effect of STIM1 RNAi on $\text{Ca}_v1.2$ function (Fig. 2B). We also coexpressed mCherry-Orai1 [exclusively PM-localized (18)] with GFP- α_{1C} in stable STIM1-expressing HEK cells, revealing areas of Orai1- $\text{Ca}_v1.2$ colocalization after store depletion (Fig. 3, L to O). Such Orai1-localization is exclusively STIM1-associated (1, 18); hence, ER-PM junctions appear to contain a complex of Orai1, $\text{Ca}_v1.2$, and STIM1 proteins.

We investigated whether STIM1 domains coupling to Orai were also coupling to α_{1C} and whether Orai might participate in STIM1- α_{1C} coupling. The smallest units of STIM1 to bind and activate Orai are the 344-442 STIM-Orai activating region (SOAR) (21) and 342-448 Ca^{2+} -activating domain (CAD) (15) (Fig. 4A). We transfected YFP-SOAR into HEK293 cells expressing α_{1C} (Fig. 4, B to F). $\text{Ca}_v1.2$ channels were activated with $\text{K}^+/\text{Sr}^{2+}$, then blocked with nimodipine, followed by Ca^{2+} addition to observe Orai function. In non-SOAR-expressing cells, there was full α_{1C} channel activity and no Orai function (Fig. 4B). In cells cotransfected with SOAR (Fig. 4C), a clear correlation existed between Orai activation, α_{1C} inhibition, and SOAR expression (Fig. 4, C and D, and fig. S4A). Cells with full α_{1C} -mediated Sr^{2+} entry had no Orai-induced Ca^{2+} entry (green), whereas cells with a large reduction in α_{1C} function showed Orai-induced Ca^{2+} entry (blue). The Orai-coupled cells had ~50% greater SOAR levels (fig. S4B); thus, SOAR coupling to both α_{1C} and Orai exactly correlated. Coexpressing Orai1-E106A with α_{1C} and SOAR (Fig. 4D) caused SOAR to be

Fig. 4. Defining the molecular domains of STIM1 mediating $\text{Ca}_v1.2$ channel inhibition. **(A)** STIM1 constructs used. **(B to D)** Fura-2 responses to $\text{K}^+/\text{Sr}^{2+}$ pulses (bars), 2 μM nimodipine, and 3 mM Ca^{2+} (arrows) in α_{1C} -expressing HEK293 cells without cotransfection (B) or with cotransfection of YFP-SOAR (C) or YFP-SOAR + Orai1-E106A-CFP (D). Statistics shown in fig. S4A. In (C), Orai-coupled ($n = 97$; cells with Orai-mediated Ca^{2+} entry) and Orai-uncoupled ($n = 135$; cells with no Orai-mediated Ca^{2+} entry) are defined in the text. Their relative YFP-SOAR expression is shown in fig. S4B. **(E)** Localization of YFP-SOAR + Orai1-E106A-CFP cotransfected cells. **(F)** Western analysis of STIM construct expression. **(G)** Fura-2 responses in α_{1C} -expressing HEK293 cells cotransfected with YFP-SOAR-LQ347/348AA alone or with Orai-E106A-CFP. **(H)** Imaging of the mutant SOAR + Orai-expressing cells in (G). **(I)** Fura-2 responses in α_{1C} -expressing HEK293 cells cotransfected with YFP-STIM1-CT alone or with Orai-E106A-CFP. **(J)** Imaging of the STIM-CT + Orai-expressing cells in (I). **(K)** Fura-2 responses in α_{1C} -expressing HEK293 cells before (left) and after (right) store depletion with 2 μM ionomycin; cells were cotransfected with either normal STIM1, the STIM1- ΔK truncation ($\Delta 667$ -685), the STIM1- $\Delta 441$ -448 deletion, or empty plasmid (statistics shown in fig. S4C). **(L)** Fura-2 responses to 1 mM Ca^{2+} addition for Orai1-CFP-expressing HEK293 cells (left) or control internal ribosomal entry site plasmid (pIRES)-HEK cells (right) transfected with YFP-STIM1-wild-type or YFP-STIM1- $\Delta 441$ -448.



more consistently PM-associated (Fig. 4E), and α_{1C} channel activity was inhibited in all cells (Fig. 4D and fig. S4A). Thus, Orai may assist SOAR associating with the PM and inhibit α_{1C} ; however, the Orai channel need not be functional to pull SOAR toward α_{1C} and inhibit α_{1C} function. Expression of the LQ347/348AA SOAR mutant (Fig. 4F) devoid of coupling to Orai1 (27) did not inhibit α_{1C} function, with or without Orai1-E106A (Fig. 4G), and did not associate with Orai1 at the PM (Fig. 4H). We expressed the STIM1 C terminus (YFP-STIM1-CT; 235-685) (Fig. 4, F, I, and J), which is largely cytosolic (15, 18). Coexpressed with Orai channels, some STIM1-CT associates with Orai1 (Fig. 4J). STIM1-CT expressed with α_{1C} caused no change in $\text{Ca}_v1.2$ activity consistent with its cytosolic location (18). However, coexpressed with Orai1-E106A, the STIM1-CT gave substantial reduction in α_{1C} activity (Fig. 4I), indicating that the association between STIM1-CT and Orai1 was sufficient for STIM1-CT to inhibit α_{1C} .

The far C-terminal K-rich region of STIM1, although not essential for Orai1 activation, assists STIM1-induced association with PM junctions (15). Compared with wild-type STIM1, STIM1 Δ K (Δ 667-685) (Fig. 4, A and F) was less effective in inhibiting α_{1C} function after store depletion (Fig. 4K and fig. S4C), indicating that the K-rich region enhances PM docking of STIM1 and association with α_{1C} . The STIM1 Δ 441-448 deletion (Fig. 4A) does not activate Orai1 channels and does not block endogenous STIM1-mediated Ca^{2+} influx (Fig. 4L) and is likely defective in Orai1-binding. However, STIM1 Δ 441-448 does still inhibit α_{1C} -mediated Sr^{2+} entry almost identical to wild-type STIM1 (Fig. 4K and fig. S4C). Thus, the site on STIM1 coupling to inhibit $\text{Ca}_v1.2$ channels is not identical to that mediating Orai channel activation. Moreover, the action of STIM1 on $\text{Ca}_v1.2$ channels does not require Orai

channel activation, despite Orai channels assisting STIM1's approach to $\text{Ca}_v1.2$ channels.

The work herein provides evidence that the $\text{Ca}_v1.2$ channel is an authentic target of STIM proteins, in addition to their well-described Orai targets. The reciprocal control mediated by STIM on the Orai and $\text{Ca}_v1.2$ channel targets may have functional implications in many cells expressing both channels. Although we reveal that the action of STIM1 on $\text{Ca}_v1.2$ channels does not require the function of Orai channels, we do reveal that the actions of STIM1 on Orai1 and $\text{Ca}_v1.2$ channels are closely connected, both spatially and functionally. Orai channels are very effective at trapping STIM1 in puncta (15) and appear to enhance STIM1 recruitment to the vicinity of $\text{Ca}_v1.2$ channels in the PM. $\text{Ca}_v1.2$ proteins are widely expressed not only in excitable cells but also nonexcitable cells—for example, immune cells including T cells, B cells, dendritic cells, and mast cells (22, 23). STIM proteins may have important roles in suppressing $\text{Ca}_v1.2$ function in immune cells, and STIM-mediated reciprocal control of $\text{Ca}_v1.2$ and Orai channels could be a decisive mechanism for controlling Ca^{2+} signals. STIM proteins are highly expressed in many excitable tissues, including smooth muscle cells (24, 25), neurons (26), and skeletal muscle (27), where they have been implicated in mediating not only Ca^{2+} signals but also fundamental control over growth, differentiation, and apoptosis (25–27). The finding of reciprocal control of the two major and widely expressed Ca^{2+} channels by a single Ca^{2+} -sensing regulatory protein should enhance understanding of Ca^{2+} signal transduction.

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Supporting Online Material

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Materials and Methods

Figs. S1 to S4

Table S1

References

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ELECTRONIC PIPETTES

Xplorer pipettes offer simply better pipetting with simplicity, precision, and reproducibility. With the Xplorer, researchers get precisely adjustable parameters, maximum reproducible results, and fatigue-free work. Besides precision and accuracy, ergonomic handling is particularly important when pipetting. With electronic piston stroke, low weight, and intuitive user guidance, electronic pipettes like the Xplorer minimize user strain. The perfect balance and the finger rest guarantee a position that is particularly easy on the joints. The basic functions can be easily chosen using a selection wheel. The Xplorer is useful for researchers who need to perform complex or long pipetting processes, without getting the many different volumes or individual steps confused.

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POSITIONS OPEN



FACULTY POSITION in Renewable Fuels at the Donald Danforth Plant Science Center

As part of the continuing expansion of the Enterprise Rent-A-Car Institute for Renewable Fuels at the Donald Danforth Plant Science Center, a faculty position at the **ASSISTANT** or **ASSOCIATE MEMBER** level is available for a scientist with research interests in the area of renewable fuels using plant or algal systems. Investigators addressing fundamental questions related to the bioenergetics, molecular biology, synthetic biology or biochemistry of biomass, hydrocarbon, or renewable feedstock production are especially encouraged to apply. Postdoctoral experience and a record of excellence in research are required. The successful candidate is expected to establish a strong, creative, and collaborative hypothesis-driven research program. Please submit curriculum vitae, a statement of research plans, and three reference letters electronically (e-mail: bcbrocker@danforthcenter.org) to: Ms. Billie Brocker, Director of Human Resources, ERAC Position, Donald Danforth Plant Science Center, 975 North Warson Road, St. Louis, MO 63132. Review of applications will begin November 30, 2010. For more information, please visit [website: http://www.danforthcenter.org/](http://www.danforthcenter.org/). The Donald Danforth Plant Science Center is an Equal Opportunity/Affirmative Action Employer. Qualified women, minorities, veterans, and individuals with disabilities are encouraged to apply.

FACULTY POSITION in Chemical Biology University of Colorado

The Colorado Initiative in Molecular Biotechnology (CIMB) at the University of Colorado at Boulder invites applications for a tenure-track faculty position in the broad area of Chemical Biology. The ideal applicant will establish an innovative research program using chemical synthesis and related methods to solve important problems in biology and medicine. CIMB is a program which integrates faculty from the departments of Chemistry and Biochemistry; Chemical and Biological Engineering; Molecular, Cellular and Developmental Biology; Ecology and Evolutionary Biology; Physics; Integrative Physiology; Applied Mathematics; Computer Science; and Mechanical Engineering ([website: http://cimb.colorado.edu/](http://cimb.colorado.edu/)). A successful candidate may be rostered in any one of these departments, and would be housed in the Jennie Smoly Caruthers Biotechnology Building, scheduled for completion in the fall of 2011. The position is at the **ASSISTANT PROFESSOR** level, although senior candidates at **ASSOCIATE** and **FULL PROFESSOR** ranks will also be considered. Candidates must have a Ph.D. degree and a demonstrated commitment to teaching at undergraduate and graduate levels, and would be expected to develop an internationally recognized research program.

Candidates interested in the position must apply at JOBSATCU posting #811526 by going to this [website: http://www.jobsatcu.com/applicants/Central?quickFind=62784](http://www.jobsatcu.com/applicants/Central?quickFind=62784). Applicants will be asked to attach separate PDF files of curriculum vitae, statements of research and teaching interests, and a list of references. In addition, please arrange to have three letters of reference sent either electronically or by hard copy to: Ms. Kim Little, 596 UCB, University of Colorado, Boulder, CO 80309-0347 (e-mail: chembiojobs@colorado.edu). Review of applications will begin on November 8, 2010, and will continue until the position is filled.

The University of Colorado is committed to diversity and equality in education and employment. See [website: http://www.Colorado.edu/ArtsSciences/Jobs/](http://www.Colorado.edu/ArtsSciences/Jobs/) for full job description.

POSITIONS OPEN



ALGAL BIOLOGIST Assistant Professor, Tenure-Track Department of Biology Colorado State University

The Biology Department is recruiting a Eukaryotic Algal Biologist at the rank of Assistant Professor, as part of an expansion of bioenergy research and teaching at CSU. We seek a broadly trained individual who uses genetic, molecular, biochemical, and cell biological approaches to study fundamental biological processes that are relevant to biofuel development. The incumbent will develop an active extramurally funded and innovative research program. This position involves research, teaching, and outreach.

Applicants must have a Ph.D. in algal biology, plant biology, or a related field. Research expertise in algal biology with publications in peer-reviewed journals is required. Postdoctoral research experience is preferred. To receive full consideration, apply online at [website: http://www.biology.colostate.edu/employment](http://www.biology.colostate.edu/employment) by November 29, 2010.

CSU is an Equal Opportunity/Affirmative Action Employer. Colorado State University conducts background checks on all final candidates.

MICROBIOLOGIST Colgate University

Colgate University seeks a tenure-stream **ASSISTANT PROFESSOR** to start fall term 2011. Completion of Ph.D. prior to or shortly after the date of hire required; teaching and postdoctoral research experience desirable. The successful candidate will team-teach in a foundation course in biology (either "Molecules, Cells, and Genes" or "Evolution, Ecology, and Diversity"), teach elective courses including microbiology, and participate in all-university programs, including the Liberal Arts Core Curriculum. The appointee will join a biology faculty deeply committed to a strong, research-oriented program involving undergraduate students and will add to this effort by offering a research tutorial in her or his area of interest; opportunities also exist to lead Colgate's unique semester-long program at the NIH. The department offers excellent teaching and research facilities, and faculty have strong support from both internal and external funding sources. Please submit an application with cover letter, curriculum vitae, transcripts, and separate statements of teaching philosophy and research interests to [website: http://www.academicjobsonline.org](http://www.academicjobsonline.org). Also, arrange to have three letters of recommendation submitted. Review of applications will begin October 22 and continue until the position is filled. We intend to begin interviewing candidates by the middle of November. Applicants with dual-career considerations can find postings of other employment opportunities at Colgate and at other institutions of higher education in upstate New York at [website: http://www.upstatenyherc.org](http://www.upstatenyherc.org). Colgate University is an Equal Opportunity/Affirmative Action Employer. Developing and sustaining a diverse faculty, staff, and student body furthers the University's academic mission.

A **POSTDOCTORAL FELLOW** position is open immediately in the University of Pittsburgh Medical Center to study breast cancer. Educational background in molecular biology and research experience using animal tumor models are preferred. Please send curriculum vitae and three reference letters to: Qihong He, Ph.D., B804-PUH, MRRC, Department of Radiology, University of Pittsburgh, 200 Lothrop Street, Pittsburgh, PA 15213. Telephone: 412-647-3088; fax: 412-647-9800; e-mail: qih5@pitt.edu. The University of Pittsburgh is an Equal Opportunity/Affirmative Action Employer.



BIOLOGICAL AND SOFT MATERIALS EXPERIMENT AND COMPUTATION RANK OPEN

DEPARTMENT OF MATERIALS SCIENCE AND ENGINEERING UNIVERSITY OF ILLINOIS AT URBANA-CHAMPAIGN

Two open-rank faculty positions, experimental and computational, are announced by the Department of Materials Science and Engineering at the University of Illinois at Urbana-Champaign. We seek exceptional candidates for tenure-track or tenured faculty positions with experimental expertise in fundamental science or engineering of biomaterials, biophysics, or related biological topics, as well as the computational study of soft matter broadly defined to include polymers, biological materials, and complex fluids. Faculty members in the Department are expected to teach undergraduate and graduate courses, and initiate and sustain a vigorous graduate research program. Applicants must provide a curriculum vita that includes their teaching experience and interests, a list of publications, and a synopsis of a proposed program of research. Candidates for tenure-track positions must have three (3) letters of reference sent directly to the department. Candidates for tenured positions must have achieved national and international recognition for their scholarship; they must include the names and contact information of at least three (3) references.

The Department has 23 faculty and more than 300 undergraduate and 160 graduate students, with highly ranked graduate and undergraduate programs. Extensive state-of-the-art experimental and computational facilities are housed on campus in the Frederick Seitz Materials Research Laboratory, the Beckman Institute, and the National Center for Supercomputer Applications.

Applicants must hold an earned doctorate in an appropriate field. Salary and rank will be commensurate with qualifications. The proposed starting date for these positions is as soon as possible after the closing date. To ensure full consideration, applications must be received prior to December 3, 2010. Interviews may take place during the application period, but a decision will not be made until after the closing date. To apply for this position, please create a candidate profile at <http://jobs.illinois.edu> and upload your letter of application and resume by **December 3, 2010**. If you do not have online access, please contact the department office for further options: **Department of Materials Science and Engineering, 1304 W. Green St., Urbana, IL 61801; Telephone: (217) 333-1440; Fax: (217) 333-2736; Email: mse@illinois.edu.**

The University of Illinois is an Equal Opportunity/Affirmative Action Employer. The administration, faculty and staff embrace diversity and are committed to attracting qualified candidates who also embrace and value diversity and inclusivity.



Tufts
UNIVERSITY

Cummings School of
Veterinary Medicine

Assistant Professor in Microbiology/ Infectious Diseases

The Division of Infectious Diseases, Department of Biomedical Sciences, Cummings School of Veterinary Medicine at Tufts University, with well developed programs in biodefense/emerging infectious diseases, is seeking applications from candidates for a faculty position at the assistant professor level. A research focus in tuberculosis with experience in aerobiology will be significant advantages. The appointee will occupy the New England Regional Biosafety Laboratory (RBL), a BSL-2/BSL-3 facility funded by the NIAID.

The Division is a dynamic group of approximately 60 faculty, scientists, laboratory and animal technicians, graduate students and administrative staff. Established NIH-funded research programs include: *E. coli* O157 and associated conditions including HUS; cryptosporidiosis; microsporidiosis; botulinum intoxication therapy; vaccine development; water/food safety and biosecurity; schistosomiasis; tularemia and tick-borne diseases; shigellosis; and *C. difficile*. (www.tufts.edu/vet/biomed/infectious_diseases.htm)

Please submit curriculum vitae, a letter describing qualifications which highlight relevant experience, and full contact information for four references to: **Saul Tzipori, Chair, Search Committee, at saul.tzipori@tufts.edu.** Application reviews will continue until the position is filled. For questions, e-mail or call **508-839-7955**.

Tufts University is an Affirmative Action, Equal Opportunity Employer.

UCLA

Microbial Evolutionary Ecologist

The Departments of Ecology and Evolutionary Biology and Microbiology, Immunology and Molecular Genetics at UCLA, together with the California NanoSystems Institute, invite applications for an open rank, TENURE-TRACK position for a microbial evolutionary ecologist. We seek conceptually oriented candidates who use emerging technologies to study the ecology and evolution of microbial communities. Representative fields of study include, but are not limited to: the determinants of microbial diversity in natural environments, the ecology and evolution of polymicrobial communities living in association with eukaryotes, ecological and evolutionary responses of microbial systems to disturbance, and the ecological and evolutionary foundations for the role of microbial communities in global nutrient cycling. Candidates must have a Ph.D. and an established track record of productivity and innovative research in microbial ecology and/or evolution. Successful candidates are expected to participate in undergraduate and graduate teaching and maintain an externally funded research program. The two departments have excellent research programs (<http://www.eeb.ucla.edu>, <http://www.mimg.ucla.edu>). The California NanoSystems Institute (CNSI; <http://www.cnsi.ucla.edu>) seeks to encourage university collaboration with industry and to enable the rapid commercialization of discoveries in nanosystems-related research including Energy, Environment, Health-Medicine, and Information Technology. UCLA also has outstanding resources, including the UC Natural Reserve System, the NSF Institute of Pure and Applied Mathematics (IPAM), the Institute of the Environment (IOE) and the Molecular Biology Institute (MBI). The position is linked to the UCLA Biosciences Initiative, which brings together leading investigators across the life sciences to catalyze interdisciplinary research, and opportunities exist to build a research focus in the candidate's area of interest including the development of a campus-wide core research center.

Review of applications will begin on **October 15, 2010** and continue until the position is filled. Applicants should submit materials online (www.eeb.ucla.edu/microevo). Please include a cover letter, curriculum vitae, statements of research and teaching interests, 2-3 most significant publications, and names and addresses of three references. Please use job number: **0830-1011-02** in all correspondence.

UCLA is an Affirmative Action/Equal Opportunity Employer with a strong institutional commitment to the achieving diversity among its faculty, students and staff. Individuals with a history of and commitment to mentoring students from underrepresented minorities are encouraged to apply.

<http://www.lssa.ucla.edu/jobs/jobinfo.php?id=98>



Renal/Cardiac Research Faculty Positions

Two ASSISTANT/ASSOCIATE PROFESSOR level positions are open in the Hypertension and Vascular Research Division of Henry Ford Hospital to complement strengths in renal/cardiac cell and molecular biology and integrative physiology. Appointments will be in the Department of Internal Medicine at Henry Ford Hospital, Detroit, MI an affiliate of the Wayne State University School of Medicine with secondary appointments in basic science departments in the School of Medicine possible. The division's 8 basic scientists bring in more than \$6 million in grant support annually (<http://www.henryford.com/hypertensionresearch>). Successful applicants will develop and maintain robust, NIH-funded research programs. Minimum requirements are a Ph.D. or M.D., 3 years of postdoctoral experience, and a strong publication record. Salaries will be commensurate with experience. Positions come with generous startup packages and benefits.

To apply submit: a current CV; contact information for 4 references; and a 1-page description of research interests via e-mail in PDF format to: Emmett Bowen at ebowen1@hfhs.org. Positions will remain open until filled.

*Henry Ford Hospital is an
Equal Opportunity Employer.*

Scientific and Clinical Positions in South Africa

The KwaZulu-Natal Research Institute for Tuberculosis and HIV (K-RITH) is seeking world-class scientists to join it in creating a groundbreaking research centre at the epicentre of the tuberculosis and HIV epidemics. Its goal is to conduct outstanding basic science research on TB and HIV and translate those scientific findings into new tools to control the deadly diseases.



A K-RITH facility is being built at the Nelson R. Mandela School of Medicine in Durban, South Africa.

We are currently recruiting for the following positions:

K-RITH INVESTIGATORS

Investigators will maintain an independent scientific programme, supervise students and technicians, and contribute to scientific advancement through scholarly publications, research presentations, and conference abstracts. Positions are available at the assistant, associate, and full investigator levels.

CLINICAL DIRECTOR

In addition to the responsibilities of a K-RITH investigator, the clinical director will develop population-, clinic-, and hospital-based studies of TB and HIV cohorts and work closely with the clinical core facility manager.

CORE FACILITY MANAGERS

Core facility managers will develop and maintain advanced instrumentation in key areas, including microbiology, immunology, pharmacology, high-throughput biology, and clinical protocol development.

All positions will be based at our state-of-the-art, 4,000-square-metre facility, which is being built on the campus of the Nelson Mandela School of Medicine in Durban, South Africa. Applicants should be eager to build a research programme that capitalizes on the unique laboratory and clinical resources at K-RITH and in and around Durban.

APPLICATION DEADLINE:

December 15, 2010

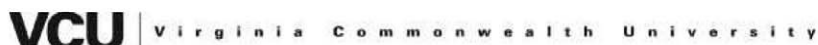
FOR MORE INFORMATION:

Go to www.k-rith.org/Science or e-mail Bronwyn Hadfield, K-RITH's human resources manager in Durban, at bronwyn.hadfield@k-rith.org.

K-RITH is a collaboration between the **Howard Hughes Medical Institute** and the **University of KwaZulu-Natal** in Durban, South Africa. Our goal is that discoveries made in the heart of the TB and HIV epidemics will drive innovation to control the deadly diseases. We know that an emphasis on basic science will lead to rational interventions and clinical solutions to many of the problems facing the area.

K|RITH

KWAZULU-NATAL RESEARCH INSTITUTE
FOR TUBERCULOSIS AND HIV



Dean, College of Humanities and Sciences

Virginia Commonwealth University (VCU) invites applications and nominations for the position of Dean of the College of Humanities and Sciences.

VCU is located on two downtown Richmond campuses — the Monroe Park Campus and the MCV Campus. As an urban institution of higher learning, VCU and its partners have contributed immeasurably to the development of the fabric of the City of Richmond, transforming it into a center of artistic and cultural growth, business development, and social services.

Virginia Commonwealth University is one of the state's largest universities and ranks among the top universities in the country in sponsored research. It enrolls over 32,000 students in 211 certificate and degree programs. MCV Hospitals and the health sciences schools of Virginia Commonwealth University compose the VCU Medical Center, one of the nation's leading academic medical centers. VCU's third campus is located in Doha, Qatar.

The College of Humanities and Sciences is dedicated to excellence in teaching, research, and public service. The College offers comprehensive undergraduate, graduate and professional programs of study, which link a foundation of understanding and knowledge with skills on which students can build careers, become responsible citizens and continue lifelong learning.

The University seeks an innovative, dynamic, and entrepreneurial individual to lead the College of Humanities and Sciences. The Dean of the College will promote disciplinary excellence and visibility while stimulating cross-disciplinary interactions and encouraging new opportunities for research, teaching, and service.

The successful candidate will be a visionary leader with demonstrated experience in Humanities and Sciences scholarship and education; proven skills in academic administration, resource management, and program formation; and a distinguished record of success in obtaining external funding. It is anticipated that candidates will have a record of excellence in scholarship and teaching that would merit a tenured faculty appointment at the rank of Professor in one of the disciplines in the College.

For best consideration, materials should be provided by **October 20, 2010**. Application materials should include a letter addressing how the candidate's experiences match the position requirements, a resume and contact information for at least five references. Submission of materials as MS Word attachments is strongly encouraged. Confidential inquiries, nominations, and application materials should be directed to: **Jan Greenwood, Greenwood/Asher & Associates, Inc; jangreenwood@greenwoodsearch.com; Phone: 850.650.2277/Fax: 850.650.2272**

Virginia Commonwealth University is an Affirmative Action/Equal Opportunity Employer, building strength through diversity. Minorities, women, veterans, and individuals with disabilities are encouraged to apply.

THREE CELL/MOLECULAR BIOLOGIST POSITIONS FORDHAM UNIVERSITY

Individuals are invited to apply for two tenure-track positions at the **ASSOCIATE PROFESSOR** level and one position at the **ASSOCIATE/FULL PROFESSOR** level in the Department of Biological Sciences, Fordham University. An ongoing grant-supported research effort is required for each position. The department has an active research program with a **Center for Cancer, Genetic Diseases, and Gene Regulation (CCGDGR)** that provides excellent research facilities, startup funds, and competitive salaries and benefits. Candidates with research interests compatible with our CCGDGR in areas such as stem cells, cancer, development, genetic diseases, bioinformatics, neuroscience, or cellular physiology are encouraged to apply. The appointees will be expected to have and maintain active research programs. A commitment to undergraduate and graduate teaching and research is required.

Applicants should e-mail one PDF application file containing a cover letter, curriculum vitae, contact information for three references, research statement, and two reprints to **thornhill@fordham.edu**. The cover letter should be addressed to **Dr. William Thornhill, Chair, Department of Biological Sciences, Fordham University, 441 E. Fordham Road, Larkin Hall 160, Bronx, NY 10458**. Candidates will be reviewed when their applications are received and we will continue to accept applications until the positions are filled.

Fordham University is an independent, Catholic university in the Jesuit tradition that welcomes applications from men and women of all backgrounds. Fordham is an EOE.

Max-Planck-Gesellschaft Max Planck Society



Max-Planck-Forschungsgruppen Max Planck Research Groups

The Max Planck Society for the Advancement of Science is an independent, non-profit research organization, whose goal is to promote top quality research at its institutes. The 80 research institutes of the Max Planck Society conduct basic research in **Biology and Medicine; Chemistry, Physics and Technology; Humanities, Social Sciences and Law**. In particular, the Max Planck Society addresses new, innovative and interdisciplinary research areas.

The Max Planck Society invites applications from outstanding young scientists in all fields of research pursued in the Society, and explicitly encourages applications from candidates with an interdisciplinary background.

The successful candidates will be offered a **Max Planck Research Group** for a period of five years at a **Max Planck Institute of their choice**. This includes a W2 position equivalent to assistant or associate professor level and a five-year grant (research positions, budget, investments). The cumulative amount of funding is competitive with top class start-up packages of international career development programs.

Applications should include a CV, a list of publications, copies of three publications, a one-page summary of scientific achievements, two letters of recommendation, and a two-page research plan.

For further information and detailed application instruction see

<http://www.mprg.mpg.de>

The Max Planck Society has established a tenure track policy for new leaders of Max Planck Research Groups; more details are available on the website above.

The Max Planck Society is committed to equal opportunities and to employing individuals with disabilities.

The deadline for application is November 17, 2010



Western University
OF HEALTH SCIENCES

The discipline of learning. The art of curing.

www.westernu.edu
www.westernu.edu

Faculty Positions in Pharmacology

College of Osteopathic Medicine of the Pacific – Northwest Lebanon, Oregon

Western University of Health Sciences, a thriving center for human health care and veterinary medicine education, is opening a new site for the College of Osteopathic Medicine of the Pacific – Northwest (COMP-NW) in Lebanon, Oregon with the inaugural class beginning in Fall, 2011. The University's 10 year plan and core values have propelled the Institution to be a benchmark University for the development of interprofessional and graduate medical education. The University values a diverse institutional community and is committed to excellence in its faculty, staff and students. Western University seeks applicants of distinguished academic accomplishments who possess a passion for excellence and can illustrate a proven track record of achievements.

The Department of Basic Medical Sciences provides the preclinical education for the College of Osteopathic Medicine, and invites applications from highly motivated individuals for tenure-track faculty positions in pharmacology. These are full-time, 12-month, tenure-track positions at the Assistant Professor/Associate Professor/Professor rank dependent upon qualifications. Successful candidates will be located at the new site on the COMP-NW campus. Applicants must have a Ph.D. in pharmacology or equivalent field and at least 2 years of postdoctoral experience. Similar positions in physiology, biochemistry/genetics, and microbiology/immunology are also available at both the Lebanon, OR and Pomona, CA campuses. Preference will be given to master educators who have demonstrated excellence in teaching with significant scholarly activity and/or those with a history of extramural funding and a strong potential to obtain further grant support for their research program. Submit a current curriculum vitae and a cover letter describing your teaching experience and philosophy, your research activity and goals, and how you meet the qualifications for the position. Please include contact information for at least three references. These positions will remain open until filled.

Nissar A. Darmani, PhD

Assistant Dean for Basic Sciences and Research

Department of Basic Medical Sciences

College of Osteopathic Medicine of the Pacific

Western University of Health Sciences

309 E. Second Street, Pomona, CA 91766-1854

Email Address: ndarmani@westernu.edu

Western University of Health Sciences is an equal opportunity employer.

Competition for Plant Scientists

The Howard Hughes Medical Institute and the Gordon and Betty Moore Foundation are collaborating on a new program to support highly promising researchers from a range of disciplines relevant to plant sciences research. HHMI and GBMF will provide flexible research support for up to 15 individuals selected as HHMI-GBMF investigators.

Eligibility:

- A doctoral degree
- Tenured, tenure-track or equivalent position at an eligible U.S. institution with a rank of assistant professor or higher
- First appointment as assistant professor or equivalent no later than December 31, 2006
- Principal investigator on one or more active, national, peer-reviewed research grants that provide at least three years of support

Application deadline:

November 9, 2010, at 3 PM ET

Application information:

www.hhmi.org/research/competitions

The Howard Hughes Medical Institute-Gordon and Betty Moore Foundation Plant Science Program is a new initiative that reflects the view that plants are vital to human health and the survival of our planet—its ecology, biodiversity, and climate. Despite the central role plants play in maintaining human health and in healthcare, basic research in the plant sciences has been historically underfunded.

HHMI and GBMF will select up to 15 HHMI-GBMF investigators who have led independent laboratories at one of the approximately 200 U.S. medical schools, universities, and research institutes that are eligible for this competition.

HHMI-GBMF investigators will receive five-year appointments to HHMI and substantial research support while remaining affiliated with their home institutions. Candidates may apply directly to HHMI; prior institutional endorsement is not required.

HHMI, a nonprofit medical research organization, plays a powerful role in advancing biomedical research and education in the United States. HHMI's flagship program in biomedical research rests on the conviction that scientists of exceptional talent, commitment, and imagination will make fundamental biological discoveries for the betterment of human health if they receive the resources, time, and freedom to pursue challenging questions.

The Gordon and Betty Moore Foundation seeks to advance environmental conservation and scientific research around the world and improve the quality of life in the San Francisco Bay Area. The Foundation's Science program aims to make a significant impact on the development of transformative scientific research, and increase knowledge in emerging fields through investment in the work of researchers and organizations at the frontiers of science.

The Howard Hughes Medical Institute is an equal opportunity employer.



**SCIENTIST/SENIOR SCIENTIST POSITION
CANCER BIOLOGY RESEARCH CENTER
SANFORD RESEARCH/USD**

The Cancer Biology Research Center (CBRC) invites applications from researchers for a full-time faculty position at the rank of Scientist or Senior Scientist within Sanford Research/USD, the research division of Sanford Health in Sioux Falls, South Dakota. A historic \$400 million gift by philanthropist Denny Sanford has allowed for expansion of Sanford Research/USD with the opening of the new Sanford Research Center (300,000 sq ft) and the establishment of a collaboration with the Sanford-Burnham Institute for Medical Research La Jolla. These provide researchers with state of the art core facilities and are enabling the creation of an integrated, world class, academic research network.

We seek an outstanding basic, translational, or clinical research scientist with an active research program in Cancer Biology. An area of particular interest is the improvement of solid tumor therapies by enhancing CD8 responses or diminishing the affect of Treg responses. Advancing basic findings in this area into treatment of head and neck cancer would be preferred but not required. Applicants must hold a PhD, DVM, MD or MD/PhD degree and have a demonstrated track record of extramural grant support and a strong publication record. Successful candidates will join the energetic and collegial research community at Sanford Research/USD, serving as a mentor to junior faculty members while advancing his/her independent research program.

Significant institutional support including modern laboratory space and state-of-the-art facilities will be provided in the new Sanford Research Center. In addition, a comprehensive compensation package will be tailored to the individual's qualifications. Candidates should submit a detailed curriculum vitae, description of research experience and future plans, and at least three letters of recommendation. Application materials should be sent to:

W. Keith Miskimins, PhD

**Director, Cancer Biology Research Center
Sanford Research/USD**

2301 East 60th Street North, Sioux Falls, SD 57117-5039

Telephone: 605-312-6104; FAX: 605-312-6071

Email: keith.miskimins@sanfordhealth.org

Sanford Health is an Equal Opportunity/Affirmative Action Employer.



Washington University in St. Louis School of Engineering & Applied Science announces significant strategic investments for multiple faculty positions in the

interdisciplinary areas of **Materials and Biological Systems Engineering**. Candidates will be considered primarily at the junior level, but senior faculty with exceptionally strong records should also apply. Joint appointments across the five engineering departments and other disciplines such as sciences, medicine and, in the case of materials, architecture will be encouraged. **Materials** research areas include: nanostructured materials, nanoparticle technology, adaptive and multi-functional materials, energy harvesting and energy storage, electrochemical processes, materials synthesis, soft materials, biomaterials, hybrid materials, nanotoxicology, and multiscale processes. **Biological Systems Engineering** applicants should be experimentalists and/or computational/theoretical scientists with strong research experience and demonstrated excellence that emphasizes the use of engineering approaches in systems biology. The Engineering School is currently building phase three of a new, multi-million dollar state-of-the-art complex to support these and other research areas of societal importance.

More detailed information can be found at: engineering.wustl.edu/facultyopenings

Women and members of groups underrepresented in engineering are especially encouraged to apply.



**TENURE TRACK FACULTY POSITION
BIOCHEMISTRY**

The Department of Biology at Tufts University invites applications for a tenure-track Assistant Professor in Biochemistry. We seek a creative scholar with primary expertise in protein or enzyme biochemistry as it applies to DNA metabolism, chromatin and chromosome structure, protein interaction networks or signaling cascades. We expect this individual to use state-of-the-art technology in protein analysis and proteomics, and to complement current research strengths in the department which include genome evolution and instability, DNA replication and repair, and cell signaling (see <http://ase.tufts.edu/biology/>). The successful candidate will develop an active externally funded research program involving graduate and undergraduate students. A clear commitment to teaching excellence at the undergraduate and graduate levels is essential. Doctoral degree and a record of research productivity are required; postdoctoral experience preferred.

Applicants should submit a cover letter, curriculum vitae, separate statements of (1) research interests and plans and (2) teaching experience and philosophy in a single PDF file to karin.murphy@tufts.edu, and have three letters of reference sent to the same address. Submission of 1-3 selected reprints is encouraged, but not required. Review of applications begins November 15, 2010, and continues until the position is filled.

Tufts University is an Affirmative Action/Equal Opportunity Employer. We are committed to increasing the diversity of our faculty. Members of underrepresented groups are strongly encouraged to apply.



**California Institute of Technology
Positions in Neuroscience**

The California Institute of Technology invites applications for two tenure-track professorial positions in the Division of Biology. A position in **Systems Neuroscience** is open to investigators studying the neural mechanisms and circuit properties that underlie behavior. A position in **Cellular and Molecular Neuroscience** is aimed at applicants whose research program concerns molecular and/or cellular aspects of physiology, behavior, or neural development. Successful applicants are expected to develop innovative research programs and should also have a commitment to undergraduate teaching. Preference will be given to candidates at the Assistant Professor level; however, consideration will also be given to more senior applicants. Appointment is contingent upon completion of Ph.D.

Please submit online application at <http://biology.caltech.edu/Positions> and include a brief cover letter, curriculum vitae, relevant publications, and a description of proposed research. Applicants should indicate for which of the two positions they wish to be considered. Instructions will be given for submission of letters of reference when you apply online. Applications will be accepted until the position is filled.

The California Institute of Technology is an Equal Opportunity/Affirmative Action Employer. Women, minorities, veterans, and disabled persons are encouraged to apply.

ARC Super Science Research Fellows (Three positions)

Faculty of Medicine, Nursing and Health Sciences

- Department of Biochemistry and Molecular Biology
- Leading international university
- Clayton campus

The Opportunity

Three ARC Super Science Fellowships are available to investigate the structure, assembly and function of bacterial protein transport machines.

The Projects: The three projects will require skills in membrane protein production and characterisation, protein structure determination, biophysical assessment of membrane proteins and measurement of functional impact in terms of host-pathogen interactions. World class infrastructure support is available for the projects which will be based at Monash University's Clayton campus. Monash University is internationally recognised for research into the structure and function of membrane proteins in bacterial pathogenesis

The Fellowships: Candidates with a PhD and having an outstanding track record in any of the research areas detailed above are encouraged to apply. The projects provide opportunities to define research directions and provide leadership within a team environment. Each Fellow will be awarded a research support budget of up to \$20k per annum and will work collaboratively with a postgraduate student protégé.

All applications should address the selection criteria. Please refer to "How to Apply for Monash jobs" below.

The University

Monash University has a bold vision - to deliver significant improvements to the human condition. Distinguished by its international perspective, Monash takes pride in its commitment to innovative research and high quality teaching and learning.

The Benefits

Remuneration package: \$80,404 - \$95,481 pa Level B (includes employer superannuation of 9%)

This role is a full-time position, however flexible working arrangements may be negotiated.

Monash offers a range of professional development programs, support for research, study and overseas work, generous maternity leave and flexible work arrangements.

Duration

Three-year appointment

Location

Clayton campus

Enquiries

See Fellowship guidelines at www.arc.gov.au/pdf/SSF1011_fundingrules.pdf

For further details contact Professor Trevor Lithgow email Trevor.Lithgow@monash.edu

For complete job description and application process visit www.monash.edu/jobs/

All applications must be received by Wednesday, 1 December 2010



FACULTY POSITIONS SCRIPPS INSTITUTION OF OCEANOGRAPHY UNIVERSITY OF CALIFORNIA, SAN DIEGO

The Oceans and Atmosphere Section of Scripps Institution of Oceanography (SIO) at the University of California in San Diego (<http://scripps.ucsd.edu>) is committed to academic excellence and diversity within the faculty, staff, and student body. We invite

faculty applications (tenure-track to tenured) to fill positions in the fields listed below. In addition to having demonstrated the highest standards of scholarship and professional activity, the preferred candidates will have experience in the arena of equity and diversity such as leadership in teaching, mentoring, research or service towards building an equitable and diverse scholarly environment. SIO is a world renowned center of marine research with approximately 200 principal investigators leading research programs on all aspects of earth, ocean and atmospheric sciences.

Successful candidates will be expected to teach classes and supervise research at both the graduate and undergraduate level. The positions require a PhD degree and a competitive record of publication, as well as evidence of the ability to conduct and fund an active research program consistent with the opportunity to have done so at this career level.

Review of applications will begin on **October 15, 2010**, and will continue until positions are filled. **"All applications and related materials should be submitted electronically via Academic Personnel Online RECRUIT at: <https://apol-recruit.ucsd.edu/>."** Applicants should send a letter including descriptions of their teaching experience, research interests, past experience and leadership in equity and diversity, a list of publications, immigration status, and the names of three potential referees, along with their complete institution address, e-mail address, phone and fax numbers.

Applicants should clearly indicate for which position(s) they are applying using the areas of interest as stated below. Questions about submission of applications may be addressed to **Cristy Whitehead at 858-534-3205, (cwhitehead@ucsd.edu)**. Salary will depend on the experience of the successful applicant and will be based on the UCSD pay scales.

Revelle Chair (RP#10-201): We invite applications at the Associate or Full Professor level for the newly created Revelle Chair in Environmental Science. This faculty position builds on the legacy of Roger Revelle as a leader in the study of global environmental change, and will be filled by an outstanding senior scientist in fields related to climate dynamics and predictability. We seek an interdisciplinary scientist and educator with demonstrated ability to mentor graduate students and junior colleagues and to provide leadership in climate-related issues within the Scripps Institution and UCSD. Applicants are encouraged from all areas of climate studies, particularly those whose research focuses on large-scale coupled interactions of the atmosphere, ocean and land surface in order to understand and predict global and regional climate variability on interannual to decadal time scales and longer. Specific areas of interest include dynamical meteorology and oceanography, the role of the ocean and atmosphere in past and present climate and the physical and chemical basis of natural and anthropogenic climate change.

Climate dynamics and predictability (RP#10-202): We invite applications to fill up to two faculty positions in climate dynamics and predictability. Specific areas of interest include dynamical meteorology and oceanography, the role of the ocean and atmosphere in past and present climate and the physical and chemical basis of natural and anthropogenic climate change. Preference will be given to scholars at the rank of Assistant Professor, but excellent candidates at other levels and in other specific areas will also be seriously considered.

Ocean imaging (RP#10-203): We invite applications to fill a faculty position in Physical Oceanography and Marine Engineering. Specific areas of interest include the development of acoustic and/or electromagnetic sensor technology for observing the ocean and its subsequent use for conducting innovative experimental work at sea. Preference will be given to scholars at the rank of Assistant Professor, but excellent candidates at other levels and in other specific areas will also be seriously considered.

Additional Oceans and Atmosphere positions (RP#10-204): We invite applications to fill up to two additional faculty positions in Atmospheric Sciences, Physical Oceanography and Marine Engineering. Specific areas of interest include collection and analysis of data, ocean-state estimation, dynamical oceanography and meteorology, and coastal and near-shore processes. Preference will be given to scholars at the rank of Assistant Professor, but excellent candidates at other levels will also be seriously considered.

UCSD is an Affirmative Action/Equal Opportunity Employer with a strong institutional commitment to excellence through diversity.

ASSISTANT or ASSOCIATE PROFESSOR SOCIAL SCIENCE & CLIMATE CHANGE – #13283 College of Agriculture and Life Sciences

Cornell University recently established a Climate Change Initiative as part of its Center for a Sustainable Future. The Center is serving to focus and integrate the growing interest across academic departments in climate change to help find solutions to this pressing world issue. As part of the Climate Change Initiative, the Center is facilitating the hiring of several faculty members over the next three years in the social sciences, humanities, biological and physical sciences, and engineering (see www.ccsf.cornell.edu for more information).

In this announcement we are seeking applications from social scientists for a tenure track position at the ASSISTANT or ASSOCIATE PROFESSOR rank. The position will involve 50% research and 50% teaching responsibilities and the individual will be expected to develop an internationally recognized and externally funded social science research program in the area of climate change. Qualified applicants from any social science field must have an established record of scholarship with clear relevance to climate change issues, and should have demonstrated ability to participate in and/or lead interdisciplinary projects with teams. Some level of grounding in the biogeochemical or ecological aspects of climate change, or a track record that demonstrates successful collaboration with these elements, will be viewed positively, as our vision is to hire someone capable of bridging social and biophysical sciences in line with the traditions of the College of Agriculture and Life Sciences. Additionally, demonstrated ability to engage non-academic audiences in industry, civil society, and government in research is expected. The scope of the position is intentionally broad and a wide range of research areas will be considered. This "open department" search seeks to place outstanding candidates in departments they best match. Potential home departments are Applied Economics and Management, Communication, Development Sociology, and Natural Resources. There is potential for a multi-departmental appointment if appointment is at the rank of associate professor.

Qualifications: Applicants should submit a cover letter indicating why they are a good candidate for this position and this Initiative, including a statement of research and teaching interests, together with a *curriculum vitae*. All materials, which may include one or two reprints, should be submitted electronically as a single PDF file to **Prof. David Wolfe, Search Committee Chair, c/o Kelly Tillotson, klt8@cornell.edu**. Three letters of recommendation are also requested. Applications will be reviewed through January 15, 2011, or until a suitable candidate is identified.

Cornell University, located in Ithaca, New York, is an inclusive, dynamic, and innovative Ivy League university and New York's land-grant institution. Applications from women and minorities are encouraged.



Cornell University
Cornell University is an Affirmative Action/
Equal Opportunity Employer and Educator.



The California Nanosystems Institute (CNSI) at the University of California- Santa Barbara is pleased to announce the competition for the **ELINGS PRIZE FELLOWSHIPS** in **EXPERIMENTAL SCIENCE**

These prize postdoctoral fellowships provide a salary of \$60,000/yr for two years, renewable for a third, along with benefits and research funds. Successful applicants will work with experimental CNSI faculty; applicants should indicate the experimental group(s) with which they would prefer to work. See www.cnsi.ucsb.edu/fellowships.

Applicants holding a PhD in science or engineering should prepare a cover letter, curriculum vitae, a one-page research proposal, and arrange for 3 supporting letters and submit via the website <https://fellow.cnsi.ucsb.edu> by **December 1, 2010**.

The University of California is an Equal Opportunity/Affirmative Action Employer.



Associate Director of Basic Sciences Faculty Leadership Position

The Hollings Cancer Center (HCC) at the Medical University of South Carolina (MUSC) invites applications and nominations for the position of HCC Associate Director of Basic Sciences. This is a senior leadership position in the Center working with the HCC Director to develop new strategies to foster collaborative and transdisciplinary interactions, mentor junior basic science investigators and identify opportunities for innovative research. The successful applicant must have an established independent research laboratory effort and demonstrable evidence of leadership ability. The academic appointment will be within an appropriate MUSC department based on the applicant's area of research.

The Hollings Cancer Center is a National Cancer Institute Designated Cancer Center with state-of-the-art research and shared resource facilities, and an extramural research portfolio of \$42M. The Center boasts more than 130 faculty scientists for which most participate in four formally organized basic science cancer research programs – Lipid Signaling in Cancer, Cancer Genes and Molecular Regulation, Developmental Cancer Therapeutics and Cancer Immunology. MUSC/HCC is looking for an individual who will complement and expand this existing expertise in cancer research and who will join the HCC's senior leadership in navigating the growth of the Center's efforts.

Located on the Atlantic coast in South Carolina, Charleston offers one of the nation's most historic downtown areas and offers a superb quality of life including easy access to ocean beaches, extensive outdoor recreation, and internationally renowned arts and cultural events. Interested individuals should e-mail their CV and a summary of future research plans to:

Andrew S. Kraft, M.D.
Director, Hollings Cancer Center
Medical University of South Carolina
MSC 955
Charleston, SC 29425
campbth@musc.edu



CHAIR Pharmacological and Pharmaceutical Sciences University of Houston College of Pharmacy

The College of Pharmacy invites qualified individuals to apply for the position of Chair of the Department of Pharmacological and Pharmaceutical Sciences. The department is co-located on the University of Houston main campus and the world-renowned Texas Medical Center. Current faculty research interests include signal transduction, drug delivery, transcellular transport and metabolism, cardiovascular and renal pharmacology, respiratory pharmacology, neuroscience, regulation of membrane transporters, GPCR structural proteomics, receptor cell biology, and natural products. For a detailed description of individual faculty research interests, please visit www.uh.edu/pharmacy. Department faculty members contribute to the Pharm.D., Ph.D. and Pharm.D./Ph.D. degree programs. Candidates must show strong evidence of academic leadership, a commitment to professional and graduate education and peer recognition as an accomplished researcher. Successful candidate is expected to have a nationally recognized and funded research program that would complement existing research programs within the department. The new Chairperson will have faculty lines to fill, and should have the ability to recruit and develop outstanding faculty. The successful candidate also may be eligible to receive one of several Endowed Professorships.

Applications will be reviewed until the position is filled. Candidates should send a letter describing research interests, administrative and educational philosophies and long-term goals, a curriculum vitae and the names, addresses (postal and e-mail), phone and fax numbers of at least three professional references in electronic (PDF) format to:

Mustafa Lokhandwala, Ph.D., Chair, Search Committee
C/O_salazar@uh.edu
University of Houston College of Pharmacy
Houston, TX 77204-5000

The University of Houston is an Affirmative Action/Equal Opportunity Employer. Minorities, women, veterans, and persons with disabilities are encouraged to apply.

UT SOUTHWESTERN MEDICAL CENTER AT DALLAS

ENDOWED SCHOLARS PROGRAM IN MEDICAL SCIENCE

Unique and highly competitive, **THE ENDOWED SCHOLARS PROGRAM IN MEDICAL SCIENCE** is designed to launch the next generation's scientific leaders on their biomedical research careers by providing seed money, research space and startup support for groundbreaking projects. The Endowed Scholars Program gives early-career investigators the chance to take risks, supported by the mentoring of experienced and highly distinguished established researchers at **The University of Texas Southwestern Medical Center at Dallas**.

As one of the foremost research institutions in the world, UT Southwestern, with four Nobel Prizes awarded to its faculty since 1985 and 18 members of the National Academy of Sciences, is poised to lead the way in a new era of scientific discovery in the 21st century. We conduct more than 3,500 research projects annually totaling more than \$400 million. Endowed Scholars have access to exceptional core facilities, which include DNA microarray services; electron microscopy; live-cell imaging; mouse gene knockouts, transgenesis and metabolic phenotyping; high-throughput chemical screening; and structural biology. UT Southwestern also is home to one of the nation's first 7-Tesla magnetic resonance imaging devices for human studies. UT Southwestern has a vibrant graduate school and nearly 4,400 medical, graduate and health professions students, residents and postdoctoral fellows.

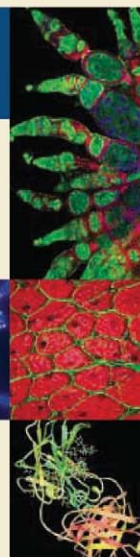
The Endowed Scholars Program, which is fully funded from private endowment, provides more than \$1,000,000 over four years to support the independent research activities, salary and benefits of each scholar. Up to five new scholars are selected each year from top universities, institutions and laboratories around the world. Each scholar is appointed as a tenure-track assistant professor in a UT Southwestern academic department or research center. Positions in both basic science and clinical departments are available.

Potential scholars submit nomination materials to a chair or director of one of UT Southwestern's basic or clinical academic departments or research centers. Those chairs or directors then forward finalists' materials for consideration to the medical center's Endowed Scholars Committee.

For detailed information about nomination materials and currently available positions, please visit our Web page: utsouthwestern.edu/utsw/home/scholars

*Applications from women and underrepresented minority candidates are strongly encouraged.
UT Southwestern is an equal opportunity institution.*

UT SOUTHWESTERN
MEDICAL CENTER



M UNIVERSITY OF MICHIGAN

BIOLOGICAL SCIENCES SCHOLARS PROGRAM

For Junior, Tenure-Track Faculty

The University of Michigan announces recruitment for the Biological Sciences Scholars Program (BSSP) to continue to enhance its investigational strengths in the life sciences research programs.

Now entering its 14th year, this Program has led to the recruitment of outstanding young scientists in the areas of genetics, microbiology, immunology, virology, structural biology, pharmacology, biochemistry, molecular pharmacology, stem cell biology, cancer biology, physiology, cell and developmental biology, and the neurosciences. The Program seeks individuals with PhD, MD, or MD/PhD degrees, at least two years of postdoctoral research experience, and evidence of superlative scientific accomplishment and scholarly promise. Successful candidates will be expected to establish a vigorous, externally-funded research program, and to become leaders in departmental and program activities, including teaching at the medical, graduate, and/or undergraduate levels. Primary college and department affiliation will be determined by the applicant's qualifications and by relevance of the applicant's research program to departmental initiatives and focus. All faculty recruited via the BSSP will be appointed at the Assistant Professor level.

APPLICATION INSTRUCTIONS: Please apply to the Scholars Program through the BSSP website at: (<http://www.med.umich.edu/medschool/research/bssp/>). A curriculum vitae (including bibliography), a three-page research plan, an NIH biosketch, and three original letters of support should all be submitted through the BSSP website. More information about the Scholars Program, instructions for applicants and those submitting letters of recommendation, and how to contact us is located on the BSSP web site: (<http://www.med.umich.edu/medschool/research/bssp/>). The deadline for applications is Friday, October 29, 2010.

The University of Michigan is an Affirmative Action/Equal Opportunity Employer.

Career opportunities in the tropics

James Cook University is one of Australia's most distinctive universities with a focus on creating a brighter future for life in the tropics worldwide. The University is located in the vibrant regional community of tropical Queensland, which has one of the fastest growing economies in Australia adjacent to the Great Barrier Reef and Wet Tropics World Heritage Areas. The University's internationally recognised research is matched by strong commitment to its region, partners and teaching.

Lecturer/Senior Lecturer – Biology (3 positions)

Ref. No. 10191 – Townsville

Employment Type: Full-time, continuing

Salary: Lecturer - Academic Level B - AUS \$73,814 - AUS \$87,096 per annum or
Senior Lecturer - Academic Level C - AUS \$89,751 - AUS \$103,033 per annum

Applications close: 29 October 2010

Staff Benefits include a generous superannuation scheme with up to 17% employer contributions, five weeks annual recreation leave, flexible working arrangements and attractive options for salary packaging.

For more information go to:
www.jcu.edu.au/jobs, enter the
Reference Number in the search field
and follow the links.

www.jcu.edu.au/jobs



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40
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California Institute of Technology Cellular, Developmental, or Regulatory Biology

The California Institute of Technology invites applications for a tenure-track professorial position in the Division of Biology. The successful applicant is expected to develop an innovative research program aimed at deciphering the molecular mechanisms that underlie biological phenomena at the level of molecules, cells, or organisms, and to be committed to high quality teaching. Preference will be given to candidates at the Assistant Professor level; however, consideration will also be given to more senior applicants. Appointment is contingent upon completion of Ph.D.

Please submit online application at <http://biology.caltech.edu/Positions> and include a brief cover letter, curriculum vitae, relevant publications, and a description of proposed research. Instructions will be given for submission of letters of reference when you apply online. Applications will be accepted until the position is filled.

The California Institute of Technology is an Equal Opportunity/Affirmative Action Employer. Women, minorities, veterans, and disabled persons are encouraged to apply.

UT SOUTHWESTERN MEDICAL CENTER

ASSISTANT PROFESSOR

The Department of Neuroscience at the University of Texas Southwestern Medical Center at Dallas, under the leadership of Dr. Joseph Takahashi, invites applications at the Assistant Professor level for a tenure-track Faculty position in the broadly defined areas of neurogenetics, electrophysiology and imaging. We seek outstanding scientists addressing molecular and genetic mechanisms underlying behavior, neural circuits and related neurological disorders. Our emphasis is on individuals using forward genetic approaches to understand the nervous system and behavior. Individuals using advanced functional approaches to study neural circuits are also particularly encouraged to apply. Scientists within the Department of Neuroscience participate in a vibrant, interdisciplinary, interdepartmental, and highly collaborative research community within the University, and enjoy access to state-of-the-art research cores in imaging, mouse MRI imaging, metabolic phenotyping, behavioral phenotyping, protein chemistry, structural biology, genomics, genetics and transgenic technology.

Applicants should submit a curriculum vitae, two-page summary of research accomplishments and future plans. Applicants should arrange to have 3-5 letters of recommendation sent to the search committee.

Please e-mail application materials to: neurosciencesearch@utsouthwestern.edu, Neuroscience Search Committee, The University of Texas Southwestern Medical Center at Dallas, 5323 Harry Hines Blvd., Dallas, TX 75390-9111. The deadline for receipt of applications is November 15, 2010.

The University of Texas Southwestern Medical Center is an Affirmative Action/Equal Opportunity Employer. Women and minority candidates are encouraged to apply.

Faculty Position Molecular Biology Sloan-Kettering Institute

The Molecular Biology Program of the Sloan-Kettering Institute, Memorial Sloan-Kettering Cancer Center (www.ski.edu), has initiated a faculty search at the Assistant Member level (equivalent to Assistant Professor). We are interested in outstanding individuals who have demonstrated records of significant accomplishment and the potential to make substantial contributions to the biological sciences as independent investigators. Successful applicants will have research interests that move the Program into exciting new areas that complement and expand our existing strengths in the areas of maintenance of genomic integrity, regulation of the cell cycle, and regulation of gene expression. Faculty will be eligible to hold appointments in the Gerstner Sloan-Kettering Graduate School of Biomedical Sciences, the Weill Cornell Graduate School of Medical Sciences, as well as the Tri-Institutional MD/PhD Training Program.

The deadline for applications is **November 1, 2010**. Interested candidates should visit <http://facultysearch.ski.edu> to access the on-line faculty application. Please visit the site as soon as possible, as it contains important information on the required application materials, including deadlines for submission of letters of reference.

Informal inquiries may be sent to Julie Kwan at kwaj@mskcc.org or to Dr. Kenneth Mariani, Chair, Molecular Biology Program at kmariani@sloankettering.edu. MSKCC is an equal opportunity and affirmative action employer committed to diversity and inclusion in all aspects of recruiting and employment. All qualified individuals are encouraged to apply.



Memorial Sloan-Kettering
Cancer Center

www.mskcc.org

CDF Group Leader/Group Leader Zebrafish Genetics



The Wellcome Trust Sanger Institute is a world leader in genomic research, with an expanding scientific programme dedicated to understanding gene function in health & disease.

The Wellcome Trust Sanger Institute invites applications from outstanding individuals with a primary research interest in zebrafish genetics to join our Faculty and lead key project work in the zebrafish area. The successful candidate will benefit from, and contribute to, the Institute's large-scale mutagenesis programmes including high throughput production and phenotyping of ENU-induced zebrafish mutants. Further details of the programmes can be found at www.sanger.ac.uk/research/areas/mouseandzebrafish/

Our Faculty members enjoy access to unparalleled facilities to support the delivery of projects and the generation of data and biological resources on a large scale. Our core technologies include high throughput sequencing, genotyping and core IT support which underpin our global position in these areas. During the coming years we aim to make a major contribution to the understanding of gene function, similar in impact to our role in genome sequencing.

We are seeking to appoint a Faculty member at either the Career Development Fellow (CDF) Group Leader and/or at the more experienced Group Leader level. Further details of our Faculty model can be found at www.sanger.ac.uk/workstudy/career/faculty/

Applicants will have a strong background in genetic research using zebrafish as a model organism, having excelled as postdoctoral scientists, career development fellows and/or junior group leaders and will be able to demonstrate that they have the capability and personal qualities to lead a research team and direct independent work, possibly for the first time.

CDF Group Leader positions are offered for a period of 6 years to scientists or clinical researchers who wish to consolidate their research skills and make the transition from postdoctoral fellow to independent scientist. They provide the opportunity to develop the intellectual basis, biological resources and data to sustain a scientific programme in the long term. At the end of the 6 year term it is expected that the post holder will pursue their career elsewhere.

For a Group Leader position applicants will typically be in their first or second group leader role, have multiple first author publications in excellent journals and be able to demonstrate strong evidence of a growing scientific reputation including references predicting their likely success as an independent investigator.

Other information

Salary from £40,000 per annum dependent on experience.

Contact Derek Stemple for formal enquiries: ds4@sanger.ac.uk

Formal applications can be made through our online recruitment facility <https://jobs.sanger.ac.uk/>

General queries can be directed to hrrservices@sanger.ac.uk

Jobs at Sanger can be followed using <http://twitter.com/sangerinstjobs>

Benefits

The Institute has excellent purpose built facilities on the Genome Campus, Hinxton on the outskirts of Cambridge. We offer a comprehensive range of benefits including a final salary pension scheme and excellent onsite facilities. Further details can be found on our website <http://www.sanger.ac.uk>

Closing date for applications is 17 October 2010.

stimulating innovative learning
skills jobs lifelong remuneration
scientific technological career
conditions competence work
flexible research passion

Europe needs researchers...

The European Commission's Joint Research Centre (JRC) is a reference centre of science and technology for the European Union. Operating in Belgium, Germany, Italy, Spain and the Netherlands, with a headcount of over 2,700, the JRC is currently seeking researchers with the right blend of competence, experience and language skills to work at the cutting edge of scientific and technological developments supporting EU policies.

If you have a sound track record of research in **Chemistry, Biology and Health Sciences, Physics, Structural Mechanics, Quantitative Policy Analysis, Spatial Sciences, Environmental Sciences, Energy Sciences or Communication/Information Technology**, you can make all the difference within the JRC's stimulating multicultural environment. In return, you can expect

a lifetime of different opportunities, a competitive remuneration package, flexible working arrangements and the chance to become involved in some of the most exciting research initiatives in Europe today.

To learn more, visit www.jrc.ec.europa.eu/competitions and as soon as you are ready to apply, fill in your on-line application at www.eu-careers.eu

Registration closes on **4th November 2010**.

References: COM/AD/01/10 – COM/AD/16/10

The JRC promotes equal opportunities and non-discriminatory practices in the workplace.





Jiangsu Academy of Agricultural Sciences

Seeking Distinguished Scientists in Various Agriculture Areas

Jiangsu Academy of Agricultural Sciences (JAAS) is a professional agricultural research and extension institution that has been established since 1932. JAAS ranks at the top of provincial agricultural academies in China in terms of the comprehensive strength in agriculture. JAAS's headquarter and main research facilities are located in Nanjing, Jiangsu, China.

Currently, there are 3 distinguished full professor positions available for application in the following areas: breeding, food processing, bioenergy, facility agriculture, and large-scale farming for modern animal husbandry. Applicants should have a faculty position already beyond the assistant professor level in a university or the equivalent position in a research institution. In addition, all candidates should demonstrate excellent records of research accomplishment and have a command of bilingual language for English and Chinese, both in spoken and written.

Successful applicants will be offered a competitive package, including sufficient laboratory space, startup funding, relocation fee and competitive salary commensurate with experience, in addition to a housing allowance, and other employee benefit. Applicants can go to www.jaas.ac.cn for application details.

In addition, more information for other regular faculty positions from JAAS relevant to a variety of disciplines in agriculture is also available at www.jaas.ac.cn.

Contact information

E-mail: rsc-gbk@jaas.ac.cn; Tel: 086-25-84390037



Invite applications for its Director of COST Office

COST (European Cooperation in Science and Technology) is one of the longest-running European instruments supporting cooperation among scientists and researchers across Europe. It provides a natural multi-national arena for such cooperation through its inter-governmental structure and by developing a partnership of trust among the COST member countries and beyond. The COST Committee of Senior Officials invited the European Science Foundation (ESF) as its implementing agent for the duration of FP7 through a contract with the European Commission. The ESF therefore provides the administrative, scientific and technical secretariat for the COST Domain Committees and COST Actions.

The European Science Foundation (ESF) provides a platform for its Member Organisations to advance European research and explore new directions for research at the European level.

Mission of the Position

The mission of the position is to support the Committee of Senior Officials (CSO) and its President in facilitating cooperation and interaction in scientific and technical research in Europe. The Director of the COST Office supports the ESF in the capacity of implementing agent of COST. This will be done by managing the COST Office and its operations within the terms of the EC/ESF contract on COST and the COST/ESF Memorandum of Understanding by developing and implementing strategies to support the COST Actions as an efficient science networking tool.

Responsibilities, profile and employment conditions are described in the complete position announcement available at www.cost.esf.org/service/jobs and www.esf.org/vacancy-DCO

The place of work is Brussels, Belgium.

Please send your application (cover letter + CV in English) by **2 November 2010** to job@cost.eu and jobs@esf.org quoting the following reference **DCO**.

The first round of interviews will be held on **16 November 2010**. There will be a **second round with selected candidates on 26 November 2010**. Both interviews will take place in Brussels, Belgium.



Research Plant Biologist/Res. Molecular Biologist/ Res. Geneticist (Plants) Plant Gene Expression Center Albany CA

Salary Range of \$81,460 - \$148,806
Announcement Open: September 20, 2010
to November 15, 2010

Applicants are invited to apply for a permanent, full-time position as a research scientist at the Plant Gene Expression Center (PGEN) of the United States Department of Agriculture (USDA), Agricultural Research Service (ARS). PGEN research is conducted through a close collaborative partnership with the University of California, Berkeley. The successful candidate is expected to develop an independent program to carry out innovative research that enhances our knowledge of plant processes, such as development and environmental response. The successful candidate's program should complement ongoing research at the PGEN, which concerns genetic and molecular analysis of disease resistance, circadian rhythms, light signaling, reproduction and development. The successful applicant will be considered for an Adjunct Faculty appointment in the Department of Plant and Microbial Biology, University of California, Berkeley. The position will be filled at the GS-12/13/14 level (Assistant/Associate Professor equivalent, salary commensurate with experience).

To apply, print a copy of vacancy announcement **ARS-X10W-0257** from the ARS Careers Website at www.ars.usda.gov/careers and follow the application directions provided. For information about the position, please e-mail **Dr. Jennifer Fletcher**, Jennifer.Fletcher@ars.usda.gov. U.S. citizenship is required. Applications must be received by the closing date of the announcement.

USDA/ARS is an Equal Opportunity Employer and Provider.



Assistant Professor of Vertebrate Ecological Physiology School of Integrative Biology University of Illinois at Urbana-Champaign

The School of Integrative Biology and the Department of Animal Biology at the University of Illinois, Urbana-Champaign, seek an outstanding scientist with a background and experience in vertebrate ecological physiology for a full-time, 9-month, tenure-track faculty position at the assistant professor level with a target start date of August 16, 2011. Candidates must have a Ph.D. or equivalent in a relevant field by start date. The successful candidate will be expected to develop an externally funded research program, teach at the undergraduate and graduate level, and collaborate with other faculty to develop research initiatives relevant to vertebrate biology.

The ideal candidate's research on physiology will interface with ecology, evolutionary biology, development, behavior, and/or molecular genetics. Incorporation of a field component in the research will be especially valued. Salary is commensurate with qualifications and experience.

Urbana-Champaign, located 120 miles south of Chicago, offers a variety of cultural opportunities that showcase the area's diverse ethnic population, superb public and private schools, quality public transportation, and a rapidly expanding community of high-tech businesses.

To ensure full consideration please create your candidate profile through <https://jobs.illinois.edu> and upload your application letter, curriculum vitae, and summary of research and plans. In addition, three letters of reference must be sent to: **Search Committee Chair, School of Integrative Biology, University of Illinois, 286 Morrill Hall, 505 S. Goodwin Ave., Urbana, IL 61801 or e-mail sib@life.illinois.edu**. All information must be received by **October 29, 2010**. For further information contact: **Vertebrate Ecological Physiology Search Committee, sib@life.illinois.edu or 217-333-3488**. Applicants may be interviewed before **October 29, 2010**; however, no hiring decision will be made until after that date.

Illinois is an Affirmative Action/Equal Opportunity Employer and welcomes individuals with diverse backgrounds, experiences, and ideas who embrace and value diversity and inclusivity. (www.inclusiveillinois.illinois.edu).



UNIVERSITY OF
LIVERPOOL



FIAT

Faculty of Science and Engineering
School of Physical Sciences
Department of Chemistry

Postdoctoral Researcher

£30,747 - £35,646 pa

You will join the research group of Professor A Hodgson to study the structure of water at interfaces. You should have (or be about to obtain) a PhD in Physics or Chemistry, with experimental expertise in surface science techniques including UHV and surface preparation. Experience in surface analysis techniques such as LEED would be advantageous. The post is available for 3 years.

Job Ref: R-573352/S Closing Date: 29 October 2010

2 Postdoctoral Researchers

£30,747 pa

To work on a 36 month multidisciplinary frontier research project supervised by Dr Darren Bradshaw, focussed on the growth and processing of porous metal-organic frameworks (MOFs) and their composites into application-specific configurations to address key societal challenges in healthcare and sustainability. You should have a PhD in Chemistry or Materials Science and a demonstrable track record of research excellence.

Post 1:

This post is central to the development of experimental protocols for the controlled nanoscale growth and solubilisation of MOFs for biomedical applications. You will have a background in biomineralisation, nanoscience, biocomposites or biological chemistry and proven skills in the handling and functionalisation (eg fluorescent tags, cell targeting groups) of protein vesicles and cages. Relevant experience in the physical characterisation of protein-based biocomposites and colloids and assessment of their stability is essential.

Job Ref: R-573316/S

Post 2:

This wide-ranging synthetic role involves the preparation and characterisation of a range of MOF-based composite systems across multiple length scales using porous polymer and oxide supports and processable biopolymer matrices for applications in catalysis and separation. A background in porous composite materials is required with one of the following areas also highly desirable: soft matter (including gels), colloids and interfaces, membranes. Relevant experience of materials characterisation (eg microscopy (SEM), diffraction methods, light scattering) to augment the synthetic effort is also essential. Job Ref: R-573317/S

Closing date for both posts: 22 October 2010

For full details, or to request an application pack, visit www.liv.ac.uk/working/job_vacancies/ or e-mail jobs@liv.ac.uk Tel 0151 794 2210 (24 hr answerphone) please quote job ref in all enquiries.

COMMITTED TO DIVERSITY AND
EQUALITY OF OPPORTUNITY



Stonewall
DIVERSITY CHAMPION



Western
University
OF HEALTH SCIENCES

The discipline of learning. The art of caring.

DEAN, GRADUATE COLLEGE OF BIOMEDICAL SCIENCES

Western University of Health Sciences (WesternU) is a rapidly growing, thriving center for graduate health care and veterinary education. WesternU's core values have propelled the University to impeccable levels of excellence.

In 2009, WesternU added four colleges – Dental Medicine, Optometry, Podiatric Medicine and Graduate Biomedical Sciences to its existing colleges of Osteopathic Medicine, Allied Health, Graduate Nursing, Pharmacy and Veterinary Medicine.

This year, WesternU completed the construction of a 178,000 square foot Health Education Center that added 26,556 square footage of new research space, and a 78,000 square foot Patient Care Center.

WesternU provides a truly innovative and medically relevant research infrastructure that brings together researchers with synergistic expertise and projects in three thematic areas that are represented by three developing centers of excellence: Center for Molecular and Metabolic Diseases, Center for Infectious Diseases and Immunology, and Center for Integrative Neurobiology.

WesternU recently embarked on a long-range program to enhance its standing as a graduate academic health science center committed to expanding its institutional research presence. The establishment of the Graduate College of Biomedical Sciences (GCBS) reflects this commitment and places WesternU in a prominent role for health-related research and training.

The GCBS was created to train highly skilled and caring biomedical researchers and future practitioners for entry into academia, industry or the medical professions. To that end, the GCBS strives to graduate humanistic professionals who will produce new biomedical knowledge and practice their healthcare professions with a deeper and broader understanding of the dynamic sciences underlying the treatment of their patients. The GCBS offers three individual programs: Master of Science in Biomedical Sciences, Master of Science in Pharmaceutical Sciences, and as of summer 2010, Master of Science in Medical Sciences. The University Strategic Plan calls for an expansion of these programs, other appropriate master degree level offerings in the biomedical sciences, and the development of innovative PhD programs in selected disciplines.

WesternU seeks an active researcher with administrative academic acumen to provide visionary leadership and strategic direction to the GCBS. The Dean will oversee the day to-day operations of all graduate programs leading to an advanced degree in the biomedical sciences within the GCBS. The selected candidate will be an individual who possesses superior leadership, management, operational, communication and external networking skills.

Position qualifications include a PhD or equivalent with a minimum of ten (10) years experience at a medical or graduate school and/or a research institute. Academic administrative experience and a successful history of recent NSF/NIH funding are required. Candidates must meet the criteria for faculty appointment at the level of professor, tenured. Additional details on the position can be found at our website: https://jobs.westernu.edu/applicants/jsp/shared/Welcome_ess.jsp. Please direct application materials to:

Dr. Philip Nelson, Chair
Dean CGBS Search Committee
Western University of Health Sciences
309 East Second Street
Pomona, CA 91766-1854
Email: pnelson@westernu.edu

Please apply by **October 31, 2010**. The position will remain open until filled.

*Western University of Health Sciences is an
Equal Opportunity Employer.*

Division of Developmental Biology and Pediatric Urology Faculty Position

Assistant Professor in Genito-Urinary Tract Development

The Divisions of Developmental Biology and Pediatric Urology are jointly recruiting for a faculty position in Genito-Urinary Tract Developmental Biology/Developmental Genetics research. Candidates must hold PhD, MD or MD/PhD degree. We seek a candidate who will develop a strong federally funded basic science program to identify the mechanisms of development of the genito-urinary system. Faculty at Cincinnati Children's Hospital Medical Center have access to high-quality graduate and MD/PhD programs and training programs for postdoctoral fellows, clinical fellows, residents and clinical faculty.

Our Children's Hospital is currently ranked number three in the country in terms of NIH funding, just behind those at Harvard and the University of Pennsylvania. Dr. Chris Wylie heads the Division of Developmental Biology, which includes about 20 primary faculty appointments, with a wide range of developmental biology research interests. Dr. Pramod Reddy heads the Division of Pediatric Urology.

For more details on these divisions, and research carried out at CCHMC, visit:

<http://www.cincinnatichildrens.org/research/div/dev-biology/default.htm>,

<http://www.cincinnatichildrens.org/ed/research/degree/mdh/default.htm>,

<http://www.med.uc.edu/pstp>.

Interested candidates should submit curriculum vitae, summary of past research accomplishments and future goals, and contact information for three references to:

Steven Potter, PhD

Search Committee Chair, ML 7007

Cincinnati Children's Hospital Research Foundation

3333 Burnet Avenue

Cincinnati, Ohio 45229-3039

genito-urinary@cchmc.org

Cincinnati Children's Hospital Medical Center is an Affirmative Action/Equal Opportunity Institution. Women and Minorities are encouraged to apply.



STANFORD
UNIVERSITY

STANFORD UNIVERSITY DEPARTMENT OF CHEMICAL AND SYSTEMS BIOLOGY

The Department of Chemical and Systems Biology at Stanford University School of Medicine invites applications for two tenure-track positions at the ASSISTANT PROFESSOR level. We are particularly interested in candidates with a strong interdisciplinary record in the broad areas of chemical biology, systems biology, and/or cellular and molecular biology in normal and disease states. Stanford offers an outstanding environment for creative interdisciplinary biomedical research. The main criterion for appointment in the University Tenure Line is excellence in research and teaching.

Candidates should have a Ph.D. and/or M.D. degree and postdoctoral research experience. Candidates should send curriculum vitae, a description of future research plans and the names and addresses of three potential referees by **November 1, 2010** to:

James Ferrell, Professor and Chair

c/o Jean Kavanagh, FAA

Department of Chemical and Systems Biology

269 Campus Drive, CCSR Bldg Room 4145A

Stanford University School of Medicine

Stanford CA 94305-5174

Stanford University is an Equal Opportunity Employer and is committed to increasing the diversity of its faculty. It welcomes nominations of and applicants from women and minority groups, as well as others who would bring additional dimensions to the university's research, teaching, and clinical missions.



WTO OMC

Organización Mundial del Comercio

La Secretaría de la Organización Mundial del Comercio (OMC) en Ginebra, Suiza, se propone proveer un puesto de traductor científico-técnico en la Sección Española de Traducción.

Funciones generales

Traducir del inglés y del francés al español documentos consistentes principalmente en notificaciones de medidas sanitarias y fitosanitarias, obstáculos técnicos al comercio, documentos referentes a la tecnología de la información y opiniones y debates de expertos sobre cuestiones técnicas y científicas que se planteen en el contexto del sistema de solución de diferencias de la OMC.

Calificaciones requeridas

Título universitario o formación equivalente en el ámbito científico y tecnológico. Se valorará especialmente la formación en biología, agronomía, veterinaria u otras ramas de especialización análogas. Experiencia y competencia demostradas en el campo de la traducción técnica. Amplia cultura general. Por lo menos tres años de experiencia práctica en la traducción. Pleno dominio del español a nivel de lengua materna. Dominio del inglés y del francés.

Para mayor información sírvase consultar el sitio Web de contratación electrónica de la OMC en la dirección: www.wto.org, **contratación-electrónica**, Aviso de vacante EXT/F/10-27

Fecha límite para la presentación de candidaturas: 26 de noviembre de 2010



The University of Texas at Austin

Stem Cell Biology Position The Institute for Cellular and Molecular Biology

The Institute for Cellular and Molecular Biology, Alan Lambowitz, Director, invites applications for a tenure-track/tenured position in Stem Cell Biology. Academic appointments at the level of Assistant, Associate, or Full Professor will be in the Section of Molecular Cell and Developmental Biology.

Candidates should have an outstanding record of research productivity and a research plan based on molecular mechanisms in any area of Stem Cell Biology. Building on a strong existing faculty, the Institute has recruited more than 50 new faculty members over the past ten years. In addition to its highly interactive and interdisciplinary research environment, the Institute provides administrative and financial support for the Graduate Programs in Cell and Molecular Biology, Microbiology, and Biochemistry, as well as state-of-the-art core facilities including DNA and next-gen sequencing, mass spectrometry, electron and confocal microscopy, DNA microarrays, robotics, and mouse genetic engineering. An MD-PhD program with the UT Medical Branch and UT-Austin's new Dell Pediatrics Research Institute enhance the environment for Biomedical Research.

Austin is located in the Texas hill country and is widely recognized as one of America's most beautiful and livable cities.

Applications received before November 1, 2010 will receive first consideration, but applications will be accepted until the position is filled. Please send a single PDF file containing your curriculum vitae, summary of research interests and names of three references to: **MCDB_stem_cell@biosci.utexas.edu**. References may also send their letters directly to the same email address.

Homepages • <http://www.biosci.utexas.edu/MCDB/> • <http://www.icmb.utexas.edu/>

The University of Texas at Austin is an Equal Opportunity Employer. Qualified women and minorities are encouraged to apply; a background check will be conducted on applicant selected.



**NORTHWESTERN
UNIVERSITY**

Faculty position in Environmental Microbiology at Northwestern University

The Department of Civil and Environmental Engineering at Northwestern University invites applications for a tenure-track or tenured faculty position in environmental microbiology. We seek outstanding candidates who are working on complex microbial systems in either engineered or natural environments. The successful candidate will have a strong background in microbiology, microbial ecology, or environmental biotechnology, an interest in cutting-edge cross-disciplinary research, and the ability to contribute substantially to our core teaching efforts spanning environmental science and engineering. Research areas of interest include developing new methods for probing complex microbial communities, improving fundamental understanding of the behavior and interactions of microbes in both natural environments (e.g., aquatic, sedimentary, and geological systems) and built environments (e.g., buildings, bioreactors, and engineered water systems), manipulating microbial metabolism to enhance desirable microbial processes in natural systems and engineered bioreactors, e.g., to remove excess nutrients or to degrade or sequester contaminants, developing novel microbiota-based technologies for sustainable energy production, and understanding, predicting, and preventing pathogen transport and the transmission of waterborne disease both in the developed world and in developing countries.

The hallmark of Northwestern is exceptionally strong, collaborative, interdisciplinary research. Excellent opportunities exist to collaborate with core faculty in Environmental Engineering and Science (described at <http://ees.civil.northwestern.edu/>), as well as more broadly with faculty in a variety of related departments, notably in Biochemistry, Molecular Biology and Cell Biology; Chemical and Biological Engineering; Biomedical Engineering; Engineering Sciences and Applied Mathematics; Earth and Planetary Sciences, and Microbiology/Immunology. Successful candidates can take great advantage of and contribute to the new One Northwestern initiative in the Life and Biomedical Sciences, which links research and educational efforts between the McCormick School of Engineering and Applied Science, the Weinberg College of Arts and Sciences, and the Feinberg School of Medicine (<http://onenorthwestern.northwestern.edu/vision>). Northwestern offers exceptionally powerful analytical facilities to all faculty through a series of core centers. Facilities for microbiology research are available on both Northwestern's main campus and at the downtown medical campus (<http://www.research.northwestern.edu/facilities/listing.html>).

Review of applications will begin in September, 2010, and the search will proceed until the position is filled. Preference will be given to applications submitted by November 30, 2010. Applications should be submitted electronically as a PDF document containing a cover letter, curriculum vitae, a two-to-three-page description of research accomplishments and plans for future work, a one-to-two page description of teaching interests, and a list of at least three persons who can provide letters of recommendation. Application materials should be submitted to the Environmental Microbiology Search Committee Chair via e-mail at jacqueline-jones@northwestern.edu, or via the web interface located at <http://facsearch.mccormick.northwestern.edu/cee/apply.php?id=26>.

It is anticipated that this position will be filled at the junior level but outstanding senior candidates will also be considered.

Northwestern University is an Affirmative Action Equal Opportunity Employer. Women and individuals in underrepresented groups are encouraged to apply. Hiring is contingent upon eligibility to work in the United States.

ETH

Eidgenössische Technische Hochschule Zürich
Swiss Federal Institute of Technology Zurich



EMPA
Materials Science & Technology

Assistant Professor (Tenure Track) of Advanced Inorganic Materials

The Department of Chemistry and Applied Biosciences at ETH Zurich (www.chab.ethz.ch) and the Empa Materials Science & Technology (www.empa.ch) invite applications for an anticipated tenure-track position at the rank of Assistant Professor for Advanced Inorganic Materials. Outstanding candidates at a more senior level will also be considered. The Department offers a stimulating environment in widespread chemical synthesis, catalysis, advanced physical and analytical methods as well as theoretical chemistry and provides first-class experimental infrastructure. The professorship is assigned to the Laboratory of Inorganic Chemistry and will have its laboratory at Empa which will provide outstanding infrastructure and unique synergy with its material science and technology teams. The candidate should have a strong and innovative research program in the fields of inorganic synthesis of solids, nano-chemistry and energy-related materials. Teaching duties involve the chemistry curriculum at the undergraduate level and advanced courses in chemistry of solids and nano particles in the Master's program. The new professor will be expected to teach undergraduate level courses (German or English) and graduate level courses (English).

Assistant professorships have been established to promote the careers of younger scientists. The initial appointment is for four years with the possibility of renewal for an additional two-year period and promotion to a permanent position.

Please submit your application together with a curriculum vitae, a list of publications, and a brief statement of present and future research interests to the President of ETH Zurich, Prof. Dr. Ralph Eichler, Raemistrasse 101, 8092 Zurich, Switzerland (or via e-mail to faculty-recruiting@sl.ethz.ch), no later than November 30, 2010. When applying electronically, do only send one PDF file. With a view towards increasing the number of female professors, ETH specifically encourages qualified female candidates to apply.



**UNIVERSITY OF CALIFORNIA, BERKELEY
ASSISTANT PROFESSOR OF
METABOLIC BIOLOGY**

The Department of Nutritional Science and Toxicology, at the University of California Berkeley, is recruiting for an assistant professor (nine-month tenure-track) starting as early as July 1, 2011.

We expect the appointee to develop a vigorous and independent research program investigating metabolic regulation, control of metabolic systems, and/or the relationship among nutrients, phytochemicals, toxicants, genetics, and disease. Applicants should have a bioscience Ph.D., M.D., or equivalent degree, with training and experience in experimental biology. The appointee will have opportunity to work with Ph.D. students in four interdepartmental programs: Molecular and Biochemical Nutrition (Metabolic Biology); Molecular Toxicology; Comparative Biochemistry; Endocrinology. The appointee also will contribute to educating undergraduates seeking degrees in nutritional biology and molecular toxicology. Moreover, the school/department is interested in candidates who will contribute to diversity and equal opportunity in higher education through their teaching, research, and service. Numerous opportunities exist for interactions with colleagues at UCB and in the Bay Area.

Applicants should provide a curriculum vitae, a statement of current and proposed research, copies of publications, and the names and addresses of at least three references. Applicants should have their referees send references to us directly, and should refer their referees to the UC Berkeley Statement of Confidentiality at: <http://apo.chance.berkeley.edu/evaltr.html>. Applications should be submitted via <http://ecnr.berkeley.edu:80/sReg.php?i=172> or ntsearch@berkeley.edu (electronic submissions strongly preferred) or to **Search Committee Chair, Department of Nutritional Science & Toxicology, 119 Morgan Hall, University of California, Berkeley, CA 94720-3104**. Consideration of applications will begin on **November 15, 2010**. The closing date for this search is: **November 30, 2010**.

The University of California is an Equal Opportunity, Affirmative Action Employer. We are especially interested in a diverse applicant pool. Berkeley is committed to addressing the family needs of faculty, including dual career couples and single parents.



**CASE WESTERN RESERVE
UNIVERSITY
SCHOOL OF MEDICINE**

**Protein Biophysics/Structural Biology Faculty Position
Department of Physiology and Biophysics
Case Western Reserve University School of Medicine
Cleveland, Ohio**

The Department of Physiology and Biophysics is undergoing major expansion. We invite outstanding individuals to apply for a faculty position at the level of Assistant Professor (tenure track), Associate Professor, or Professor. The major focus of the current search is the area of protein biophysics and/or structural biology, with a special emphasis on studies of membrane proteins. We especially encourage applicants who are using interdisciplinary approaches to work on basic or translational aspects of human diseases—particularly in the cardiovascular, renal, or neuronal systems. Visit our website at <http://Physiology.case.edu> or <http://Biophysics.case.edu>. The Department and School have excellent infrastructure, particularly in x-ray crystallography and NMR spectroscopy (see <http://Pepec.case.edu> and <http://Ccsm.case.edu>)

Applicants for a position as Assistant Professor should have a Ph.D. and/or M.D. degree, 3-5 years postdoctoral experience, and a strong record of scholarly activity. Competitive candidates for associate professor should have a substantial publication record and a national reputation. Competitive candidates for professor should have achieved records of leadership in the profession and have a strong record of scholarly publications.

Applicants should submit a cover letter and a full *Curriculum Vitae*. Applicants for Assistant Professor position should also include a brief description of their research plans as well as the contact information for three professional references. Please submit application materials with separate file attachments by email to: **Walter F. Boron, M.D., Ph.D., Chairman, Physiology/Biophysics Search@case.edu**.

In employment, as in education, Case Western Reserve University is committed to Equal Opportunity and Diversity. Women, veterans, members of underrepresented minority groups, and individuals with disabilities are encouraged to apply.

NC STATE UNIVERSITY

**Governor Robert W. Scott
Distinguished Professorship
Department of Chemistry**

The Department of Chemistry at North Carolina State University invites nominations and applications to fill the Governor Robert W. Scott Distinguished Professorship in chemistry. Preliminary inquiries are also encouraged. This position is one component of a major growth plan at the University with emphasis on interdisciplinary research related to life sciences and energy. The successful candidate must have a nationally and internationally recognized research program and be able to provide dynamic leadership in his or her area of research. All candidates are expected to have strong interest and ability in teaching at both the undergraduate and graduate levels. Formal requirements include a PhD in chemistry or in a related scientific field plus an established track record of accomplishments appropriate for appointment as a tenured full-professor in chemistry.

Candidates should submit an electronic copy of their curriculum vitae along with other material describing future directions of their research at: <http://jobs.ncsu.edu> under position number **101743**. Nominations and all inquiries should be sent to the Chemistry Department Chair **Morteza Khaleedi@ncsu.edu**. After a preliminary review, candidates will be contacted and asked to request letters of recommendation. However, if a candidate prefers, letters of recommendation may also be sent at the time of application and mailed to the **Governor Scott Search Committee Chair, Department of Chemistry, North Carolina State University, Raleigh, NC 27695-8204**. The review of applicants will begin on **November 1, 2010**, and will continue until candidates are selected.

We welcome the opportunity to work with candidates to identify suitable employment opportunities for spouses or partners. AA/EOE. In addition, NC State welcomes all persons without regard to sexual orientation. Persons with disabilities requiring accommodations in the application and interview process please call (919) 515-3148.



**HARVARD
School of Public Health**

**Department of Environmental Health
Assistant/Associate Professor of Environmental Epigenetics**

The Department of Environmental Health at the Harvard School of Public Health (HSPH) seeks candidates for the position of assistant or associate professor of environmental epigenetics. This is a tenure-ladder position, with academic rank to be determined in accordance with the successful candidate's experience and productivity.

We seek an outstanding scientist whose research focus is the experimental and genomic analysis of epigenetic mechanisms that modify disease susceptibility. We especially welcome candidates interested in how epigenetic alterations form the molecular basis for health effects of environmental exposures.

This tenure track position offers outstanding scholarly and scientific resources in a collegial and collaborative atmosphere. A joint appointment with Harvard Medical School and an affiliated teaching hospital is also possible. The position offers the opportunity to mentor exceptional students and postdoctoral trainees with strong interests in environmental and molecular epidemiology. The successful candidate will be expected to develop an independent research program, to participate in teaching graduate-level courses, and to supervise graduate students and post-graduates. Applicants must have a Ph.D., M.D. or equivalent graduate degree.

Please send a letter of application, including a statement of current and future research interests, curriculum vitae, sample publications, and the names of four referees to the following address. Applicants should ask their four referees to write independently to this address. The electronic submission of application documents to the email below is welcome. **Chair, Search Committee for Assistant/Associate Professor of Environmental Epigenetics, c/o B. Zuckerman, Harvard School of Public Health, Department of Environmental Health, 665 Huntington Avenue, Building 1, Room 1301, Boston, MA 02115; Email: bzuckerm@hsph.harvard.edu**

Harvard University is committed to increasing representation of women and minority members among its faculty and particularly encourages applications from such candidates.



Nontraditional Careers: Opportunities Away From the Bench Webinar

Produced by the *Science*/AAAS Business Office.

Want to learn more about exciting and rewarding careers outside of academic/industrial research? View a roundtable discussion that looks at the various career options open to scientists across different sectors and strategies you can use to pursue a nonresearch career.

**Now Available
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www.sciencecareers.org/webinar



Yale

FACULTY POSITION AT YALE UNIVERSITY

Department of Molecular, Cellular and Developmental Biology

The Department of Molecular, Cellular and Developmental Biology at Yale University invites applications for a faculty appointment as an assistant, associate or full professor from individuals working on any area of plant biology. The Department encourages applications from candidates using any experimental approach, including molecular, genetic, biochemical, or genomic/proteomic approaches, to investigate outstanding questions in modern plant biology at the organismal, cellular or molecular level. We expect the successful candidate to develop an active research group, be an interactive member of the faculty and participate in interdisciplinary research and training. The successful candidate should demonstrate communication skills commensurate with excellence in teaching at both the undergraduate and graduate levels.

Applications will close **December 10, 2010**. Submit an application, a curriculum vitae and a description of research interests on line to <https://academicjobsonline.org/ajo/Yale>. Request at least three individuals submit letters of recommendation on your behalf to <https://academicjobsonline.org/ajo/Yale>. For questions, please e-mail mcdm.plantsearch@yale.edu.

Yale University is an Affirmative Action/Equal Opportunity Employer. Women and members of underrepresented minority groups are especially encouraged to apply.



Cancer Science Institute of Singapore

Faculty and Postdoctoral Fellow Recruitment



CSI Singapore, National University of Singapore, is a state-of-the-art research center with a multifaceted and coordinated approach to cancer research, extending from basic cancer studies all the way to experimental therapeutics. The focus of the institute is on cancers endemic to Asian populations such as gastric, liver, lung, leukemia and breast cancers. The institute houses a full spectrum of research and clinical translational facilities. This major center is led by a team of world-class scientists with significant government funding from Singapore's National Research Foundation and Ministry of Education.

We are seeking individuals with exceptional scientific credentials to join a vibrant, dynamic team at CSI Singapore.

Principal Investigators

Qualified candidates at Professor, Associate Professor and Assistant Professor levels should be at the cutting edge of their field with an established record of excellence in cancer research. Candidates should have the capability and experience of leading high-level, innovative research programs resulting in international quality publications. Successful candidates can look forward to stable funding for up to 5 years commensurate with their abilities, ample laboratory space and resources, a competitive salary package and a dynamic working environment. Candidates with research emphasis in gastric, liver and lung cancers will be strongly considered.

Postdoctoral Fellows

Postdoctoral Fellow candidates should be highly-motivated, creative individuals with an outstanding PhD degree in a related discipline. Successful candidates can look forward to working with top cancer researchers in an internationally represented environment.

Interested candidates should send their CV and the names of three referees to:

Professor Daniel G. Tenen

Director, Cancer Science Institute of Singapore (CSI Singapore), NUS

Email: csi_careers@nus.edu.sg

Please indicate the position you are applying for in the subject title.

Visit our website at www.csi.nus.edu.sg



AAAS is here – helping scientists achieve career success.

Every month, over 400,000 students and scientists visit ScienceCareers.org in search of the information, advice, and opportunities they need to take the next step in their careers.

A complete career resource, free to the public, *Science Careers* offers a suite of tools and services developed specifically for scientists. With hundreds of career development articles, a grants and scholarships database, webinars and downloadable booklets filled with practical advice, a community forum providing real-time answers to career questions, and thousands of job listings in academia, government, and industry, *Science Careers* has helped countless individuals prepare themselves for successful careers.

As a AAAS member, your dues help AAAS make this service freely available to the scientific community. If you're not a member, join us. Together we can make a difference.

To learn more, visit aaas.org/plusyou/sciencecareers



The University of Porto invites applications for three INVITED CHAIRS IN BIODIVERSITY RESEARCH

The three Invited Chairs will be located at the Faculty of Sciences of Porto and be part of CIBIO (Research Centre in Biodiversity and Genetic Resources), a Center of Excellence in biodiversity, environment and evolutionary research. CIBIO conducts research in different areas, including ecology, evolution, conservation biology, behavioral ecology and landscape planning, among others (<http://cibio.up.pt>). We seek outstanding, innovative applicants with a record of excellence in research who will complement our existing interests and strengths.

The three Invited Chairs are supported in part by private or public companies and the Portuguese Fundação para a Ciência e a Tecnologia (www.fct.mctes.pt) and will last for a period of three to five years. They are (1) the BES - Invited Chair in Biodiversity, (2) the EDP - Invited Chair in Biodiversity, and (3) the REFER - Invited Chair in Biodiversity (for further details please go to <http://cibio.up.pt>). The second is a full-time job while the two others are half-time, and all are provided with generous research support, including funds for personnel, equipment and running expenses.

The successful candidates must have at least five years postdoctoral experience and are expected to develop a strong, independent research program that integrates both environmental and evolutionary aspects, to attract external funding, and contribute to graduate and undergraduate teaching. CIBIO and the University of Porto offer a stimulating scientific and cultural environment, including a variety of other research centers in the fields of ecology, evolution and the environment thus providing multiple opportunities for collaborative projects.

Application packages should include a full Curriculum vitae, a vision statement of proposed research interests and the names and addresses of three potential referees. Documents should be submitted by email, not later than **31st October 2010**, to cibio.up@mail.icav.up.pt.



Faculty Position in the *Gene Expression and Regulation Program* at The Wistar Institute

The Wistar Institute, an independent, non-profit research institute with a primary focus on cancer research, seeks an outstanding candidate with a doctoral degree or equivalent for a faculty position at any academic rank in the Gene Expression and Regulation Program. We are seeking a candidate to complement the Institute's strength on chromatin and transcription regulation. The successful candidate will use chemical, biochemical or genomic approaches within the biological context of disease such as cancer and age-associated disorders. Specific areas of focus include: chromatin regulatory mechanisms, chemical biology as it relates to chromatin enzymology, structure/biophysics of higher-order chromatin, biological models of chromatin regulation, nuclear organization of transcription and non-coding RNA regulatory mechanisms.

The Wistar Institute, an NCI-designated Cancer Center, offers highly competitive start-up support, salary and fringe benefits, in addition to a superb and interactive research environment, including state-of-the-art core facilities with multiple laboratories that are increasingly emphasizing systems biology approaches to basic biological processes, cancer and other human diseases. The Institute's location on the University of Pennsylvania campus and adjacent to Drexel University provides potential academic and clinical collaborators, as well as opportunities for training graduate students.

Applications will be reviewed as received and will be accepted until the position is filled. To ensure timely consideration, applicants should submit applications before November 1, 2010. The application should include a curriculum vitae, a brief summary of past and future research interests, history of research funding support (if applicable), and three letters of reference. Applications should be sent by e-mail to: **Ronen Marmorstein, Search Committee Chair, c/o Maria Colelli** (colelli@wistar.org), The Wistar Institute, 3601 Spruce Street, Philadelphia, PA 19104. EOE/AA/M/F/D/V.



THE WISTAR INSTITUTE

An NCI-designated Cancer Center

For more information about us, visit our Web site at
www.wistar.org



Director Aiken, SC

The University of Georgia (UGA) is searching for a successful and creative leader to guide its world-renowned Savannah River Ecology Laboratory (SREL) to new heights of accomplishment. Due to its location on the Department of Energy's protected Savannah River Site (DOE-SRS), SREL has unique access to large, undisturbed areas and unparalleled opportunities for interdisciplinary research in ecological and environmental areas of topical importance. SREL, established by ecology pioneer Eugene Odum, has many long-term data sets that are unique resources for cutting-edge environmental and ecological work in areas such as climate change, the nuclear energy renaissance, and contamination and remediation of wetlands. The next leader will develop new initiatives and help build critical partnerships with other institutions – including the Savannah River National Laboratory, other research groups on the SRS, and the Southeastern Universities Research Association (SURA) – to conduct research on and off the SRS.

The SREL Director will report directly to the UGA Vice President for Research (OVPR) and be located at SREL, but will have a faculty appointment through an appropriate UGA school or department.

The successful candidate will have a PhD, at least 15 years of experience in a relevant science, and be a nationally recognized research scientist with a strong research track record and history of external funding. Salary will be commensurate with experience. Applicants should submit their curriculum vitae and the names and addresses of at least four references by **October 31, 2010** to: **Dr. Rebecca Sharitz, Savannah River Ecology Laboratory, Drawer E, Aiken, SC 29802; Sharitz@srel.edu**.

The University of Georgia is committed to increasing the diversity of its faculty and strongly encourages applications from individuals in under-represented groups. The University is an EEO/AA institution.

UC DAVIS

Inorganic Materials Chemistry Faculty Position in the Energy for the Future Initiative at the University of California, Davis

The UC Davis Department of Chemistry invites applications for an Assistant Professor of Chemistry associated with the UC Davis Energy for the Future Initiative targeting major energy issues facing California and the nation. The Energy for the Future Initiative brings a total of fourteen new faculty positions to the campus, including a total of four positions in the Chemistry Department. The online application link is available on the Chemistry website (<http://www.chem.ucdavis.edu/>).

We seek a colleague who will synthesize new inorganic solid state compounds, characterize their structures, properties, and/or stability, with the fundamental goal of understanding the inorganic solid state at the level of atoms and chemical bonds, and the practical goal of finding new materials for energy applications. He/she will develop an independent research program and also take advantage of specialized techniques and major instrumentation, including the Peter A. Rock Thermochemistry Laboratory, TEM and spectroscopy at UC Davis, as well as synchrotron and neutron based experimentation. A competitive candidate will demonstrate an interest in actively participating in the UC Davis Energy Institute, as well as demonstrating a strong commitment to undergraduate and graduate teaching. A Ph.D. or equivalent degree in chemistry or a related discipline is required. The position is open until filled. To ensure full consideration, online applications should be submitted no later than **November 1, 2010**, for a targeted start date of **July 1, 2011**.

*The University of California is an
Affirmative Action/Equal Opportunity Employer.*

Boston University School of Medicine

Boston University School of Medicine welcomes applications for its departments below at ranks of Instructor, Assistant & Associate Professor, or Professor.

- Anatomy & Neurobiology
- Biochemistry
- Microbiology
- Physiology & Biophysics
- Pharmacology & Experimental Therapeutics
- Pathology

Email a cover letter specifying your department of interest with your CV to busmdean@bu.edu.

Boston University is an equal opportunity and affirmative action employer



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Research
Microbiologist,
GS-0403-12/13

Salary Range of \$68,809 to \$106,369

The Food Safety and Enteric Pathogens Research Unit in the National Animal Disease Center in Ames, Iowa is seeking a qualified Research Microbiologist to conduct research describing microbial communities in the swine intestinal tract. Incumbent develops alternatives to antibiotics for controlling intestinal colonization by the foodborne pathogen *Salmonella*. NADC is the premier research institute within the USDA for studying diseases of large animals and has recently undergone a \$500 million upgrade of laboratory facilities. Resources for the position include flow cytometry, mass spectroscopy, laser capture microdissection microscopy, Roche FLX, Solexa-Illumina and AP3100 sequencers, an electron microscopy/histology facility, bioinformatics resources, and both conventional and gnotobiotic large animal support. A Ph.D. in microbiology or a related field is required. Assigned research requires a professional knowledge of microbial genetics, diversity, biochemistry, and pathogenesis; metagenomic analyses of microbial ecosystems; and a working knowledge of culturing anaerobic bacteria. For further information, contact **Dr. Thad Stanton** at Thad.Stanton@ars.usda.gov. Questions regarding application procedures can be directed to **Kim Grandon** at 515-337-7277. For details and application directions, visit the website: www.ars.usda.gov/careers and refer to announcement number ARS-X10W-0263. U.S. citizenship is required. Applications must be received by **October 22, 2010**.

USDA/ARS is an Equal Opportunity Employer and Provider.

POSITIONS OPEN

ASSISTANT PROFESSOR
Neuroscientist

Department of Pathology and Anatomy
Eastern Virginia Medical School

The Department of Pathology and Anatomy at Eastern Virginia Medical School invites applicants at the Assistant Professor level for teaching Neuroscience to students in the medical, physician assistant, surgical assistant, and Ph.D. programs. Experience teaching gross anatomy and/or pathology would be helpful. No laboratory space is associated with this position. The Department has a distinguished record of teaching accomplishments. The candidate must have post-doctoral training in the neurosciences, a record of teaching medical students, and demonstrated proficiency in the use of technology in education and teaching. The position requires scholarly activity related to innovations in education or collaborative research with basic and clinical neuroscientists in the fields of, e.g., sleep or neural plasticity. There are opportunities for collaborative research with the Department of Psychiatry in the fields of animal models and psychopharmacology. Scholarly activities should lead to national/international presentations, peer-reviewed publications, curriculum innovation, and extramural funding. The position also requires service on institutional committees, student mentoring, and participation in the nationally recognized community service efforts of the school.

Applicants should send curriculum vitae, a two-page summary of teaching and research accomplishments, and the names and contact information of three to five potential references by January 15, 2011. Please mail all application materials to:

Nancy's Search Committee
Department of Pathology and Anatomy
Eastern Virginia Medical School
700 West Olney Road
Norfolk, VA 23507

EVMS is an Affirmative Action/Equal Opportunity Employer as well as a Drug and Tobacco Free Work Place.

Department of Environmental Health at Indiana University

The Department of Environmental Health, one of four academic departments in the School of Health, Physical Education, and Recreation (HPER) at Indiana University - Bloomington is forming the necessary prerequisites to transition HPER to an accredited School of Public Health. Four tenure/tenure-track positions are available through a competitive national search with anticipated hire dates of August 1, 2011. All searches will remain open until positions are filled.

Assistant Professor positions include Environmental Molecular Toxicologist and Environmental Toxicologist. Assistant-Associate-Full positions in Environmental Health Epidemiologist and Occupational Health.

Interested applicants are encouraged to peruse the HPER website: <http://www.hper.indiana.edu/about/careers.shtml> to find detailed information regarding these positions. Send letter of application, statement of professional objectives, proposed research agenda, curriculum vitae, and list of three references for junior-level positions and six references for senior-level positions to: **Chair, Search Committee, c/o Christina Lirot, clirot@indiana.edu**.

Indiana University is an Equal Employment Affirmative Action Employer committed to excellence through diversity.

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ASSISTANT PROFESSOR of BIOLOGY

The Department of Biology at California State University San Bernardino invites applications for a tenure-track position at the rank of Assistant Professor in the area of Cellular Plant Physiology. The successful applicant will develop an independent research program, and is expected to excel in teaching core courses related to cell and plant biology at the undergraduate and M.S. levels. Candidates must have a record of published research and show potential for developing and sustaining an independent, externally funded research program involving both undergraduate and M.S. students. Candidates must have a Ph.D. in the biological sciences; postdoctoral experience is desirable. Application deadline is November 30, 2010, or until position is filled. Submit curriculum vitae, statement of research accomplishments and goals, statement of teaching philosophy, and three letters of reference to: **Dr. David Polcyn, Chair, Attn: Cellular Plant Physiologist Search, Department of Biology, Cal State San Bernardino, 5500 University Parkway, San Bernardino, CA 92407. Website: <http://biology.csusb.edu/jobs.php>.**

ASSISTANT PROFESSOR Quantitative Analysis and Modeling of Natural Resources

Purdue University invites outstanding candidates with interest in such areas as ecological statistics, ecosystem modeling, and environmental analyses to apply for an academic-year, tenure-track faculty position. Visit website: <http://www.fnr.purdue.edu> for details. Submit a cover letter, including the names and contact information for three references, curriculum vitae, summary of research interests, statement of teaching philosophy and interests, and list of relevant coursework by November 15 to: **Search Chair, Purdue University, Department of Forestry and Natural Resources, 715 W. State Street, W. Lafayette, IN 47907-2061. Telephone: 765-496-2215, e-mail: bpjanow@purdue.edu. Purdue University is an Equal Opportunity/Equal Access/Affirmative Action Employer fully committed to achieving a diverse workforce.**

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UCSD | Department of Bioengineering Jacobs

FACULTY POSITION in Bioengineering (at any level, with preference for Assistant Professor)

The Department of Bioengineering Engineering (<http://be.ucsd.edu>) at the University of California, San Diego, invites applications from candidates engaged in innovative research related to multiple areas of bioengineering for one or more faculty positions. Successful applicants will be expected to teach undergraduate (especially courses in the Biotech and the BioSystems Engineering Major tracks) and graduate courses in Bioengineering and to establish a vigorous program of high-quality federally funded bioengineering research, particularly research that focuses on:

- genome-scale or systems approaches to biological complexity
- experimental investigations of dynamics of living systems, including optical methods
- integration across multiple scales from molecule to organism to neurons, and
- applications relevant to regenerative medicine

Exceptional candidates in other areas will also be given consideration. The Bioengineering Department within the Jacobs School of Engineering at UC San Diego is committed to building an excellent and diverse faculty, staff, and student body. In addition to having demonstrated the highest standards of scholarship and professional activity, or for junior scholars to have the potential thereof, the preferred candidates will have experience or demonstrated contributions to a climate that supports equity, inclusion and diversity. Applicants are asked to submit materials online at <https://apol-recruit.ucsd.edu/apply>. Please include a cover letter, a curriculum vitae including a complete list of publications, a statement summarizing research interests and teaching experience, past or planned contributions to diversity and the names of five referees with their contact information. For inquiries, please contact: **Chair, Department of Bioengineering, 9500 Gilman Drive, Mail Code 0412, La Jolla, CA 92093-0412**, or by e-mail to recruit@bioeng.ucsd.edu. Review of applications will begin immediately and continue until the position or positions are filled.

Salaries are commensurate with qualifications and experience.

UCSD is an Equal Opportunity/Affirmative Action Employer with a strong institutional commitment to the achievement of excellence and diversity <http://diversity.ucsd.edu>. For applicants interested in spousal/partner employment, please visit the UCSD Partner Opportunities Program website <http://academicaffairs.ucsd.edu/cifices/partnerecp/>.

POSITIONS OPEN

METABOLOMICS FACULTY POSITION

Institute for Cell Engineering &
Biological Chemistry
The Johns Hopkins University
School of Medicine

The Institute for Cell Engineering (ICE) and the Department of Biological Chemistry (BC) at The Johns Hopkins University School of Medicine invite applications for a tenure-track faculty position at any level. ICE and BC are jointly seeking candidates with an outstanding record in the area of metabolomics, and a commitment to excellence in research and teaching. Applicants should submit (preferably as a single PDF file), curriculum vitae, a list of publications, and a summary of research and future plans by December 15, 2010. Electronic files should be sent to **e-mail: metabolomics@jhmi.edu**. Applicants should also request that three letters of recommendation be sent electronically (preferred) or to the address below:

Gerald W. Hart, Ph.D.
Committee Chair
C/O Ms. Angelina Hines
Department of Biological Chemistry
The Johns Hopkins University
School of Medicine
725 North Wolfe Street
Baltimore, M.D. 21205-2185

Equal Opportunity/Equal Affirmative Action Employer.

BIOCHEMISTRY/BIOORGANIC CHEMISTRY and ANALYTICAL CHEMISTRY FACULTY OPENINGS

The Department of Chemistry and Biochemistry at the University of Maryland, Baltimore County (UMBC) invites applications for two tenure-track faculty positions in the areas of biochemistry or bioorganic chemistry and analytical chemistry. Applicants with research programs in any sub-field(s) of these two disciplines are welcome; however, in the latter case expertise in mass spectrometry is preferred. The appointments commence August 2011. The positions, which require a Ph.D., are anticipated to be filled at the ASSISTANT PROFESSOR level. Successful candidates are expected to establish strong research programs and teach at both the undergraduate and graduate (Ph.D., M.S.) levels. The Department is a highly cross-disciplinary and interactive group of faculty, postdoctoral fellows, Ph.D., M.S., and undergraduate students engaged in cutting edge research at the molecular level, working in excellent laboratory facilities in a recently renovated building. The University is strategically situated on a suburban campus in the intellectually and culturally vibrant Baltimore-Washington corridor, which enhances the dynamic educational and research opportunities afforded by its diversity, intermediate size and world-class infrastructure. Applicants should submit curriculum vitae, description of research plans, and statements of teaching philosophy; and arrange for three letters of recommendation to be sent to Chair, (Biochemistry/Bioorganic Chemistry or Analytical Chemistry) Faculty Search Committee at: **Department of Chemistry and Biochemistry, University of Maryland, Baltimore County, 1000 Hilltop Circle, Baltimore, MD 21250**. Electronic submissions can be made to **e-mail: chemsearch@umbc.edu**. UMBC is an Equal Opportunity/Equal Affirmative Action Employer; and applications from women, minorities, and individuals with disabilities are especially encouraged. Applications will be considered until the positions are filled.

POSTDOCTORAL POSITION to study the cytoskeletal organization of bacterial RNA processing systems using molecular biological, cell biological, and biochemical techniques. Experience in protein or membrane biochemistry will be an asset but is not a requirement. NIH supported, 2010-2014. Relevant papers: *Proc. Natl. Acad. Sci.* 104:1667, 2007; *J. Biol. Chem.* 283:13850, 2008; *J. Bacteriol.* 192:3222, 2010. Respond to: Professor L. Rothfield, Department of Molecular, Microbial and Structural Biology, University of Connecticut Health Center, Farmington, CT 06032, USA. **E-mail: lroth@neuron.uconn.edu**. The University of Connecticut is an Equal Opportunity Employer.

POSITIONS OPEN

BRADLEY UNIVERSITY

ASSISTANT PROFESSOR Department Of Biology

The Department of Biology invites applications for a tenure-track position in Microbiology at the Assistant Professor level to begin fall 2011. For additional information about the University, visit **websites: <http://www.bradley.edu> and <http://www.bradley.edu/humanresources/opportunities/faculty.shtml>**.

A Ph.D. is required, postdoctoral experience is preferred. The successful applicant will teach upper level major's microbiology, courses in his/her area of specialization, preferably immunology, general introductory biology courses, and contribute to the broader Biology curriculum. The candidate is expected to establish a productive, funded research program consistent with the University's emphasis on student-faculty scholarship collaboration and working with students of diverse backgrounds. Applicants must demonstrate a strong commitment to undergraduate teaching and research, and the promise of scholarly and pedagogical excellence in a liberal arts setting.

Qualified candidates should submit a letter of application addressing qualifications for the position, latest curriculum vitae, graduate and undergraduate transcripts, statements of teaching interests and research plans, and three professional letters of reference to:

Dr. Erich K. Stabenau, Chair
Biology Department
Bradley University
Peoria, IL, 61625

To ensure full consideration, hard copies of application materials should be received by November 15, 2010. Review of applications will begin immediately and continue until position is filled.

Bradley University is an Equal Opportunity/Equal Affirmative Action Employer. The administration, faculty and staff are committed to attracting qualified candidates from underrepresented groups.

ASSOCIATE DIRECTOR for Primate Resources New England Primate Research Center Harvard Medical School

The New England Primate Research Center of Harvard Medical School seeks qualified candidates for the position of Associate Director for Primate Resources. Successful candidates will have a D.V.M. or equivalent degree and advanced training in an appropriate discipline. The Associate Director for Primate Resources will direct all aspects of the Center's animal and veterinary care services and will manage a large, diverse, AAALAC-accredited research program involving over 200 affiliated and collaborative scientists and a permanent staff of over 60 personnel including six veterinarians. Candidates should have evidence of outstanding leadership and experience in the utilization of nonhuman primates in models of human disease.

The Division of Primate Resources supports a diverse biomedical research portfolio with a focus on genetics, infectious diseases and neuroscience. Opportunities for collaborative and independent research are available and encouraged. For detailed responsibilities and minimum qualifications, please see **website: <http://www.hms.harvard.edu/neprc/>**.

Send applications to: **Dr. Roger Speakman, Chair, Search Committee, Harvard Medical School, New England Primate Research Center, P.O. Box 9102, Southborough, MA 01772-9102**. Applications should include curriculum vitae, a concise summary of past accomplishments and future plans, and the names and contact information for at least three references. Applications will be considered until the position is filled.

Harvard Medical School and the New England Primate Research Center are Equal Opportunity Employers, committed to diversity in the workplace.

POSITIONS OPEN

ASSISTANT PROFESSOR Biological Chemistry The Johns Hopkins University School of Medicine

The Department of Biological Chemistry at The Johns Hopkins University School of Medicine invites applications for a tenure-track faculty position at the Assistant Professor level. The Department is seeking candidates with an outstanding record in any area of biochemistry, cellular, or molecular biology and a commitment to excellence in research and teaching. Applicants should submit (preferably as a single PDF file) curriculum vitae, a list of publications and a summary of research and future plans by November 15, 2010. Electronic files should be sent to **e-mail: bcrecruit@jhmi.edu**. Applicants should also request that three letters of recommendation be sent electronically or to the address below:

Craig Montell, Ph.D.
Committee Chair
C/O Ms. Claire Brzezczko
Department of Biological Chemistry
The Johns Hopkins University
School of Medicine
725 North Wolfe Street
Baltimore, M.D. 21205-2185

Equal Opportunity/Equal Affirmative Action Employer.

FACULTY POSITION in Fisheries and Mariculture

The University of Texas at Austin, Marine Science Institute invites applications from internationally recognized scientists currently of associate or full Professor rank for the position of **ASSOCIATE DIRECTOR** of Fisheries and Mariculture. The successful candidate will hold a tenured senior faculty appointment in the Department of Marine Science and while Associate Director would hold the Perry R. Bass Endowed Chair in Fisheries and Mariculture.

The Associate Director will be expected to develop an outstanding broad-based research program focused on Gulf of Mexico fishes and be responsible for: (1) managing the Fisheries and Mariculture Laboratory personnel and facilities which occupy 33,000 square feet of buildings on 10 acres of land; (2) managing the institutional funds appropriated to this program; (3) raising external funds to initiate new research that involves graduate students; (4) effectively communicating major regional and national issues in fisheries and mariculture to the general public and how they are being addressed by current research at the facility; and (5) performing the normal duties of a faculty member including teaching and University service.

Details of the position, the Institute, and application procedure are available at **websites: <http://www.utmsi.utexas.edu/hr> and <http://facultyjobs.utexas.edu>**.

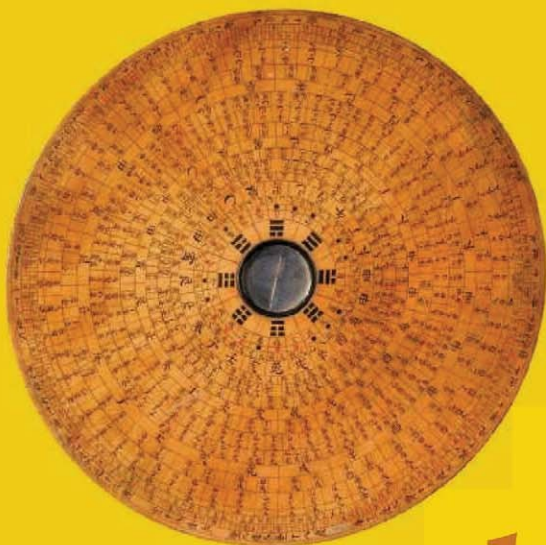
The University of Texas at Austin values diversity and is committed to Equal Opportunity and Equal Affirmative Action. Women and minorities are encouraged to apply. Background check conducted on applicant selected.

POSTDOCTORAL POSITION Department of Human Genetics Emory University School of Medicine

Postdoctoral position open in the laboratory of **Dr. Stephen Warren** for a talented, creative, and scientifically adventurous individual with documented research accomplishments and bench skills. Our work covers all aspects of Fragile X syndrome, a common inherited form of cognitive deficiency and autism (see **website: <http://genetics.emory.edu>**). We operate as a dynamic, fast-paced team focused upon discovery and seek motivated applicants with a Ph.D. and/or M.D. degree with training in neurobiology, molecular biology, and/or genetics. Experimental skills in neuronal cell culture, protein translation, histone modification, and/or iPSC cell culture would be useful. Send curriculum vitae and the contact information for three references to **e-mail: christi.bell@emory.edu**. Emory University is an Equal Opportunity/Equal Affirmative Action Employer.

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you see
all kinds
of things
you can’t
see from
the center.”

We’re now inviting world-class researchers tackling ambitious research questions to apply for Wellcome Trust Investigator Awards. These extend our fellowship funding model to researchers in posts salaried by their universities or research institutes, and are available to those based in the UK, the Republic of Ireland and low- or middle-income countries.



Chinese compass

Kurt Vonnegut

POSITIONS OPEN

FACULTY POSITION Microbial Pathogenesis

Applications are invited for faculty positions in microbial pathogenesis at the Public Health Research Institute, an infectious diseases research center at New Jersey Medical School-UMDNJ in Newark, New Jersey. Applicants with an interest in emerging infectious disease pathogens and bio-defense with expertise in host-pathogen interactions are particularly encouraged to apply. The successful candidate will have demonstrated research productivity with existing funding, and he/she will be expected to maintain an independent, innovative NIH-funded research program. A competitive startup package and outstanding core facilities are available, including animal imaging, informatics, and extensive BSL-3/ABSL-3 containment facilities, including a new Regional Biocontainment Laboratory.

Applicants should submit curriculum vitae, a statement of research experience, a summary of future plans, and names of three references by December 15, 2010, to: **Dr. Issar Smith, e-mail: smithis@umdnj.edu. Website: <http://www.phri.org>.**

Equal Opportunity Employer, Minorities/Females/Persons with Disabilities/Veterans.

PROFESSOR OF PRACTICE in Geographic Information Systems, Tulane University

The Department of Earth and Environmental Sciences seeks to fill a non-tenure-track, Professor of Practice position to teach courses in Geographic Information Systems (GIS), and to supervise the GIS computer lab. To be considered, the applicant must have experience using geologic, topographic, and geophysical raster and point cloud data sets. Expertise with ESRI products is required, including customization of GIS applications. We seek an individual possessing an enthusiastic dedication to teaching who is willing to make a long-term commitment to the department and the University. A Ph.D. is required at the time of appointment. The initial appointment will be for three years with the possibility of renewal after a performance review at the end of the second year. The deadline for applications is October 30, 2010, but the position will remain open until filled. Applications should include curriculum vitae, a statement of teaching interests and goals, and the names and contact information of at least three references, and should be sent to: **Dr. Stephen Nelson, Department of Earth & Environmental Sciences, Tulane University, 6823 Street Charles Avenue, New Orleans, LA 70118-5698. E-mail: snelson@tulane.edu preferred.** Further information about the Department and University can be obtained at [website: http://tulane.edu/sse/eens](http://tulane.edu/sse/eens). *Tulane University is an Equal Opportunity Employer. Women and minorities are encouraged to apply.*

POSTDOCTORAL FELLOWSHIPS in Immunobiology of Normal and Neoplastic Lymphocytes

This NIH sponsored program offers training in a wide variety of cancer related areas of immunology including immune recognition, regulation, and tolerance; lymphocyte development; cell signaling and adhesion; and cancer immunotherapy. Our [website: https://webdev.med.upenn.edu/contribute/t32/](https://webdev.med.upenn.edu/contribute/t32/) describes the research interests of the 31 primary trainers in the program.

Positions are open immediately for Ph.D. graduates and for those who expect to graduate in the next six months. *Applicants must be American citizens or permanent residents.*

Interested candidates should send curriculum vitae and names of three references to: **Elizabeth Thompson, Administrative Assistant, Department of Pathology and Laboratory Medicine, 616 BRB II/III, 421 Curie Boulevard, Philadelphia, PA 19104-6160. Fax: 215-573-6725. E-mail: teliza@exchange.upenn.edu.** *Women and minorities are urged to apply. The University of Pennsylvania is an Equal Opportunity/Equal Opportunity Employer.*

POSITIONS OPEN

CHIEF Section of Gastroenterology Department of Medicine Boston University School of Medicine

The Department of Medicine at Boston University School of Medicine and Boston Medical Center is recruiting a Chief of the Section of Gastroenterology. The Gastroenterology Section is composed of a very accomplished group of academic faculty and fellows, and the successful candidate would build upon a strong academic program that provides dynamic clinical care and training in the context of a remarkably diverse patient population. The successful candidate's experience and qualifications are expected to meet those for the rank of Professor. We seek an individual with a distinguished record of investigative achievement, clinical and educational excellence, and exemplary leadership skills. The Department of Medicine is one of the country's leading research-intensive departments of medicine, and the candidate would possess a well-established research program that will complement the ongoing research in the section. The ideal candidate would also contribute to research programs that exist throughout the department, medical school, and broader Boston University environment, taking advantage for instance of a superb cancer center, nanoscience/nanomedicine initiative, bio-engineering, molecular imaging center, rich genetic epidemiology resources (e.g. the Framingham Heart Study), and health services research programs, to name a few.

Interested applicants should submit a letter of interest and curriculum vitae to: **Richard A. Cohen, M.D., Chair GI Section Chief Search Committee at Vascular Biology Section, Boston University Medical Center, 650 Albany Street X720, Boston, MA 02118.** *Candidates from all backgrounds are encouraged to apply. Boston University School of Medicine is an Equal Opportunity/Equal Opportunity Employer.*

ASSISTANT PROFESSOR OF BIOLOGY

The Department of Biology at California State University San Bernardino invites applications for a tenure-track position at the rank of Assistant Professor in the area of Animal Physiology. The successful applicant will develop an independent research program, and is expected to excel in teaching core courses related to organismal and human physiology at the undergraduate and M.S. levels. Candidates must have a record of published research and show potential for developing and sustaining an independent, externally funded research program involving both undergraduate and M.S. students. Candidates must have a Ph.D. in the biological sciences; postdoctoral experience is desirable. Application deadline is November 30, 2010, or until position is filled. Submit curriculum vitae, statement of research accomplishments and goals, statement of teaching philosophy, and three letters of reference to: **Dr. David Polcyn, Chair, Attn: Animal Physiologist Search, Department of Biology, Cal State San Bernardino, 5500 University Parkway, San Bernardino, CA 92407. Website: <http://biology.csusb.edu/jobs.php>.**

YALE UNIVERSITY Department of Chemistry

The Department of Chemistry at Yale University invites applications for tenure-track positions at the ASSISTANT PROFESSOR level to commence 1 July 2011. We seek creative teacher-scholars who show promise for developing outstanding research programs and we will consider applications in any area of chemistry. Particular foci this year will be in the areas of inorganic and physical chemistry. Applicants should send their curriculum vitae, a statement of research plans, and arrange for the submission of three letters of recommendation. All materials should be received by 1 November 2010. Send applications to: **Chair, Junior Faculty Search Committee, Department of Chemistry, Yale University, P.O. Box 208107, New Haven, CT 06520-8107.** This search remains subject to final university approval. *Yale University is an Equal Opportunity/Equal Opportunity Employer and applications from women and underrepresented minority group members are especially encouraged.*

POSITIONS OPEN

FACULTY POSITIONS University of Virginia

The Department of Pharmacology at the University of Virginia seeks to fill two tenure-eligible positions. Rank is dependent upon qualifications. The first position seeks candidates investigating how ion channels relate to physiological systems or pathological processes. Areas of interest include, but are not limited to, molecular genetics and/or cell biology of ion channel regulation (expression, posttranslational modification) in the context of integrated cardio-respiratory, endocrine or neuronal network function. The second position seeks candidates exploring genetic networks, signal transduction pathways, and mechanisms involved in regulation of metabolism and the underlying pathologies of atherosclerosis, diabetes, obesity, or hypertension. Areas of interest include, but are not limited to, transcriptional regulation, signal transduction networks important for metabolic control, and the role of inflammation in the pathology of metabolic disease. The successful candidates will join growing groups of investigators utilizing molecular and physiological approaches to study ion channel biology or metabolism with an emphasis on the identification and validation of new drug targets. Candidates exploring integrative approaches including in vitro, in vivo, and/or systems biological models using cutting-edge technology are preferred. Both positions require a Ph.D., M.D., or equivalent. Faculty will enjoy superb resources including state-of-the-art core facilities, a generous startup package, and a strong collaborative research environment. To apply, candidates should visit Jobs@UVA online at [website: https://jobs.virginia.edu](http://jobs.virginia.edu). For the first position, how ion channels relate to physiological systems or pathological processes, search on posting number **0606166**; for the second position, exploring genetic networks, signal transduction pathways and mechanisms involved in regulation of metabolism and the underlying pathologies of atherosclerosis, diabetes, obesity or hypertension, search on posting number **0606266**. For either position, candidates must complete a Candidate Profile online, attach a cover letter, curriculum vitae, two-page statement of research goals and interests, and contact information for three references. The positions will remain open until filled. Questions about the positions or the application process may be directed to **e-mail: pharmsearch@virginia.edu.**

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MARKETPLACE

Recombinant Human Proteins^{rh}
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AAAS Early Career Award for Public Engagement with Science

Nominations are open **now through October 15** for the **AAAS Early Career Award for Public Engagement with Science**.

With this new award, AAAS will recognize early-career scientists and engineers who demonstrate excellence in their contribution to public engagement with science activities. The award recipient will receive a monetary prize of \$5,000, a commemorative plaque, and complimentary registration and reimbursement of travel expenses to the 2011 AAAS Annual Meeting in Washington, D.C.

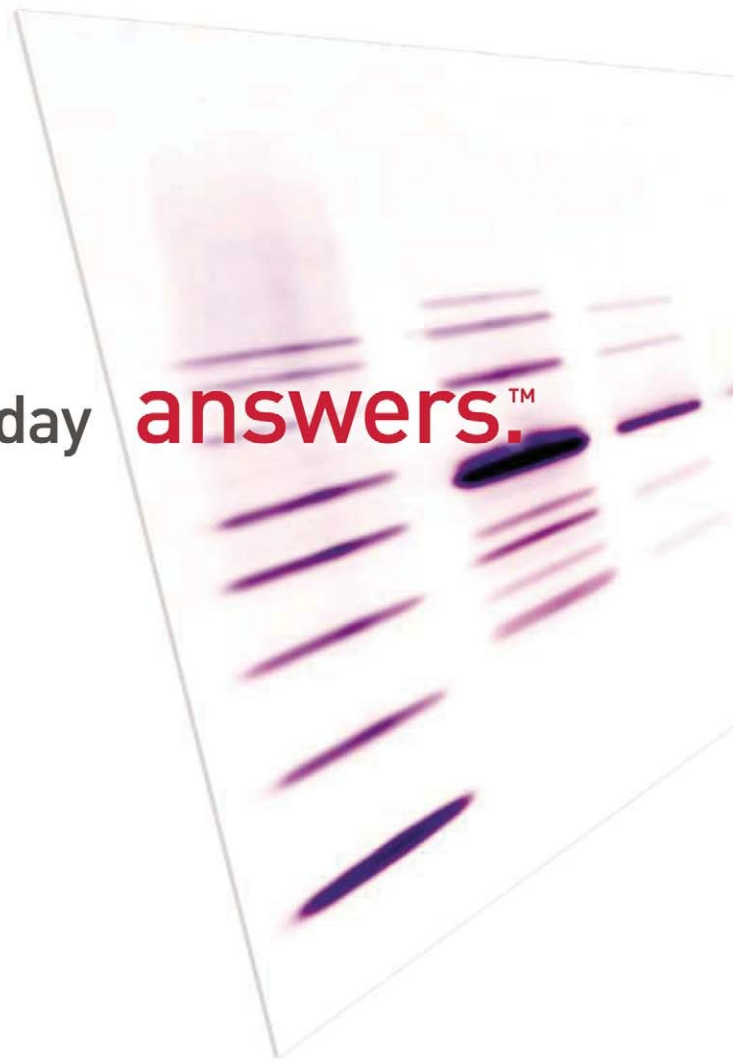
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